CHAPTER **EIGHTEEN**

# 18

# The Cell-Division Cycle



"Where a cell arises, there must be a previous cell, just as animals can only arise from animals and plants from plants." This statement, which appears in a book written by German pathologist Rudolf Virchow in 1858, carries with it a profound message for the continuity of life. If every cell comes from a previous cell, then all living organisms—from a unicellular bacterium to a multicellular mammal—are products of repeated rounds of cell growth and division, stretching back to the beginnings of life more than 3 billion years ago.

A cell reproduces by carrying out an orderly sequence of events in which it duplicates its contents and then divides in two. This cycle of duplication and division, known as the **cell cycle**, is the essential mechanism by which all living things reproduce. The details of the cell cycle vary from organism to organism and at different times in an individual organism's life. In unicellular organisms, such as bacteria and yeasts, each cell division produces a complete new organism, whereas many rounds of cell division are required to make a new multicellular organism from a fertilized egg. Certain features of the cell cycle, however, are universal, as they allow every cell to perform the fundamental task of copying and passing on its genetic information to the next generation of cells.

To explain how cells reproduce, we have to consider three major questions: (1) How do cells duplicate their contents—including the chromosomes, which carry the genetic information? (2) How do they partition the duplicated contents and split in two? (3) How do they coordinate all the steps and machinery required for these two processes? The first question is considered elsewhere in this book: in Chapter 6, we discuss how DNA is replicated, and in Chapters 7, 11, 15, and 17, we describe how

the eukaryotic cell manufactures its numerous other components, such as proteins, membranes, organelles, and cytoskeletal filaments. In this chapter, we tackle the second and third questions: how a eukaryotic cell distributes—or *segregates*—its duplicated contents to produce two genetically identical daughter cells, and how it coordinates the various steps of this reproductive cycle.

We begin with an overview of the events that take place during a typical cell cycle. We then describe the complex system of regulatory proteins called the *cell-cycle control system*, which orders and coordinates these events to ensure that they occur in the correct sequence. We next discuss in detail the major stages of the cell cycle, in which the chromosomes are duplicated and then segregated into the two daughter cells. At the end of the chapter, we consider how animals use extracellular signals to control the survival, growth, and division of their cells. These signaling systems allow an animal to regulate the size and number of its cells—and, ultimately, the size and form of the organism itself.

## QUESTION 18–1

Consider the following statement: "All present-day cells have arisen by an uninterrupted series of cell divisions extending back in time to the first cell division." Is this strictly true?

## OVERVIEW OF THE CELL CYCLE

The most basic function of the cell cycle is to duplicate accurately the vast amount of DNA in the chromosomes and then to segregate the DNA into genetically identical daughter cells such that each cell receives a complete copy of the entire genome (**Figure 18–1**). In most cases, a cell also duplicates its other macromolecules and organelles and doubles in size before it reproduces; otherwise, each time a cell divided, it would get smaller and smaller. Thus, to maintain their size, proliferating cells coordinate their growth with their division. We return to the topic of cell-size control later in the chapter; here, we focus on cell division.

The duration of the cell cycle varies greatly from one cell type to another. In an early frog embryo, cells divide every 30 minutes, whereas a mammalian fibroblast in culture divides about once a day (**Table 18–1**). In this section, we describe briefly the sequence of events that occur in proliferating mammalian cells. We then introduce the cell-cycle control system that ensures that the various events of the cycle take place in the correct sequence and at the correct time.

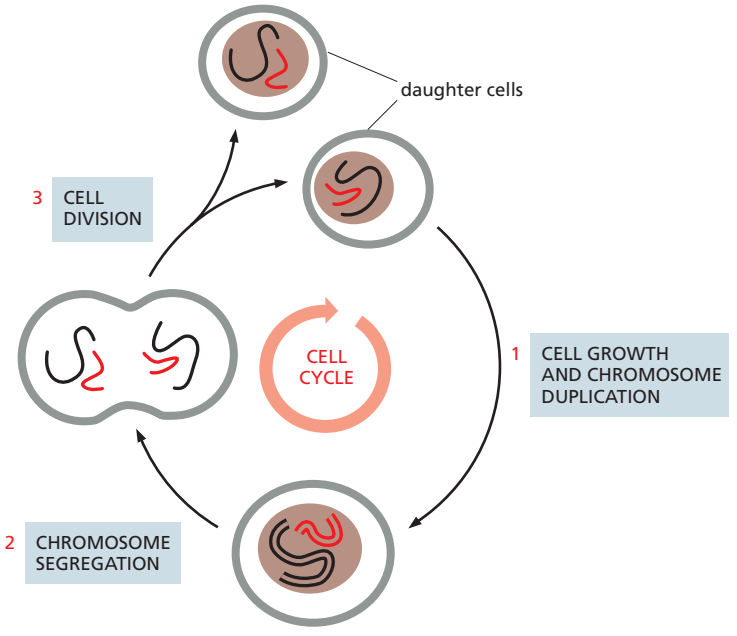



**Figure 18–1 Cells reproduce by duplicating their contents and dividing in two in a process called the cell cycle.** For simplicity, we use a hypothetical eukaryotic cell—which has only one copy each of two different chromosomes—to illustrate how each cell cycle produces two genetically identical daughter cells. Each daughter cell can divide again by going through another cell cycle, and so on for generation after generation.

| TABLE 18–1 SOME EUKARYOTIC CELL-CYCLE DURATIONS | |
|---|---|
| **Cell Type** | **Duration of Cell Cycle** |
| Early fly embryo cells | 8 minutes |
| Early frog embryo cells | 30 minutes |
| Mammalian intestinal epithelial cells | ~12 hours |
| Mammalian fibroblasts in culture | ~20 hours |

## The Eukaryotic Cell Cycle Usually Includes Four Phases

Seen in a microscope, the two most dramatic events in the cell cycle are when the nucleus divides, a process called *mitosis*, and when the cell itself then splits in two, a process called *cytokinesis*. These two processes together constitute the **M phase** of the cycle. In a typical mammalian cell, the whole of M phase takes about an hour, which is only a small fraction of the total cell-cycle time (see Table 18–1).

The period between one M phase and the next is called **interphase**. Viewed with a microscope, it appears, deceptively, as an uneventful interlude during which the cell simply increases in size. Interphase, however, is a very busy time for a proliferating cell, and it encompasses the remaining three phases of the cell cycle. During **S phase** (S = synthesis), the cell replicates its DNA. S phase is flanked by two "gap" phases—called **$G_1$ phase** and **$G_2$ phase**—during which the cell continues to grow (**Figure 18–2**). During these gap phases, the cell monitors both its internal state and external environment. This monitoring ensures that conditions are suitable for reproduction and that preparations are complete before the cell commits to the major upheavals of S phase (which follows $G_1$) and mitosis (following $G_2$). At particular points in $G_1$ and $G_2$, the cell decides whether to proceed to the next phase or pause to allow more time to prepare.

During all of interphase, a cell generally continues to transcribe genes, synthesize proteins, and grow in mass. Together with S phase, $G_1$ and $G_2$ provide the time needed for the cell to enlarge and to duplicate its cytoplasmic organelles. If interphase lasted only long enough for DNA replication, the cell would not have time to double its mass before it divided and would consequently shrink with each division. Indeed, in some special circumstances that is exactly what happens. In an early frog embryo, for example, the first cell divisions after fertilization (called *cleavage divisions*) serve to subdivide the giant egg cell into many smaller cells

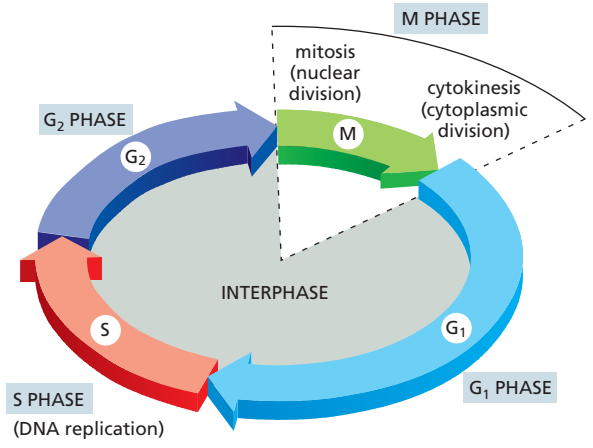
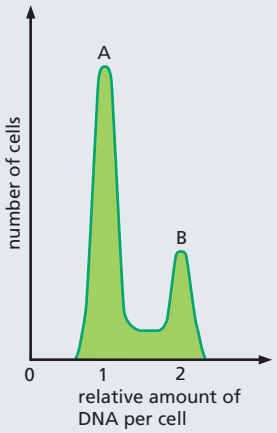



**Figure 18–2 The eukaryotic cell cycle usually occurs in four phases.** The cell grows continuously during interphase, which consists of three phases: $G_1$, S, and $G_2$. DNA replication is confined to S phase. $G_1$ is the gap between M phase and S phase, and $G_2$ is the gap between S phase and M phase. During M phase, the nucleus divides in a process called mitosis; then the cytoplasm divides, in a process called cytokinesis. In this figure—and in subsequent figures in the chapter—the lengths of the various phases are not drawn to scale: M phase, for example, is typically much shorter and $G_1$ much longer than shown.

## QUESTION 18–2

A population of proliferating cells is stained with a dye that becomes fluorescent when it binds to DNA, so that the amount of fluorescence is directly proportional to the amount of DNA in each cell. To measure the amount of DNA in each cell, the cells are then passed through a flow cytometer, an instrument that measures the amount of fluorescence in individual cells. The number of cells with a given DNA content is plotted on the graph below.




Indicate on the graph where you would expect to find cells that are in $G_1$, S, $G_2$, and mitosis. Which is the longest phase of the cell cycle in this population of cells?

as quickly as possible (see Table 18–1). In such embryonic cell cycles, the $G_1$ and $G_2$ phases are drastically shortened, and the cells do not grow before they divide.

## A Cell-Cycle Control System Triggers the Major Processes of the Cell Cycle

To ensure that they replicate all their DNA and organelles, and divide in an orderly manner, eukaryotic cells possess a complex network of regulatory proteins known as the *cell-cycle control system*. This system guarantees that the events of the cell cycle—DNA replication, mitosis, and so on—occur in a set sequence and that each process has been completed before the next one begins. To accomplish this organizational feat, the control system is itself regulated at certain critical points of the cycle by feedback from the process currently being performed. Without such feedback, an interruption or a delay in any of the processes could be disastrous. All of the nuclear DNA, for example, must be replicated before the nucleus begins to divide, which means that a complete S phase must precede M phase. If DNA synthesis is slowed down or stalled, mitosis and cell division must also be delayed. Similarly, if DNA is damaged, the cycle must be put on hold in $G_1$, S, or $G_2$ so that the cell can repair the damage, either before DNA replication is started or completed or before the cell enters M phase. The cell-cycle control system achieves all of this by employing a set of molecular brakes, sometimes called *checkpoints*, to pause the cycle at certain transition points. In this way, the control system does not trigger the next step in the cycle unless the cell is properly prepared.

The cell-cycle control system regulates progression through the cell cycle at three main transition points (**Figure 18–3**). At the transition from $G_1$ to S phase, the control system confirms that the environment is favorable for proliferation before committing to DNA replication. Cell proliferation in animals requires both sufficient nutrients and specific signal molecules in the extracellular environment; if these extracellular conditions are unfavorable, cells can delay progress through $G_1$ and may even enter a specialized resting state known as $G_0$ (G zero). At the transition from $G_2$ to M phase, the control system confirms that the DNA is undamaged and fully replicated, ensuring that the cell does not enter mitosis unless its DNA is intact. Finally, during mitosis, the cell-cycle control machinery

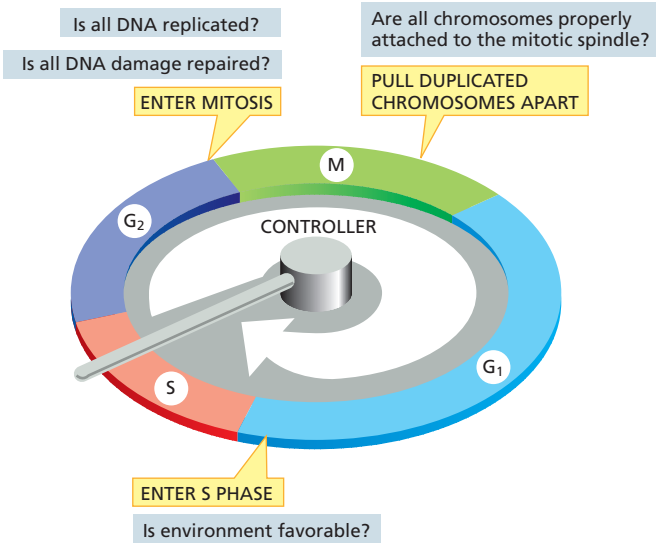



**Figure 18–3 The cell-cycle control system ensures that key processes in the cycle occur in the proper sequence.** The cell-cycle control system is shown as a controller arm that rotates clockwise, triggering essential processes when it reaches particular transition points on the outer dial. These processes include DNA replication in S phase and the segregation of duplicated chromosomes in mitosis. The control system can transiently halt the cycle at specific transition points—in $G_1$, $G_2$, and M phase—if extracellular or intracellular conditions are unfavorable.

ensures that the duplicated chromosomes are properly attached to a cytoskeletal machine, called the *mitotic spindle*, before the spindle pulls the chromosomes apart and segregates them into the two daughter cells.

In animals, the transition from $G_1$ to S phase is especially important as a point in the cell cycle where the control system is regulated. Signals from other cells stimulate cell proliferation when more cells are needed—and block it when they are not. The cell-cycle control system therefore plays a central part in the regulation of cell numbers in the tissues of the body; if the control system malfunctions such that cell division is excessive, cancer can result. We discuss later how extracellular signals influence the decisions made at the $G_1$-to-S transition.

### Cell-Cycle Control Is Similar in All Eukaryotes

Some features of the cell cycle, including the time required to complete certain events, vary greatly from one cell type to another, even within the same organism. The basic organization of the cycle, however, is essentially the same in all eukaryotic cells, and all eukaryotes appear to use similar machinery and control mechanisms to drive and regulate cell-cycle events. The proteins of the cell-cycle control system first appeared more than a billion years ago, and they have been so well conserved over the course of evolution that many of them function perfectly when transferred from a human cell to a yeast (see How We Know, pp. 30–31).

Because of this similarity, biologists can study the cell cycle and its regulation in a variety of organisms and use the findings from all of them to assemble a unified picture of how the cycle works. Many discoveries about the cell cycle have come from a systematic search for mutations that inactivate essential components of the cell-cycle control system in yeasts. Likewise, studies of both cultured mammalian cells and the embryos of frogs and sea urchins have been critical for examining the molecular mechanisms that underlie the cycle and its control in multicellular organisms like ourselves.

## THE CELL-CYCLE CONTROL SYSTEM

Two types of machinery are involved in cell division: one manufactures the new components of the growing cell, and another hauls the components into their correct places and partitions them appropriately when the cell divides in two. The **cell-cycle control system** switches all this machinery on and off at the correct times, thereby coordinating the various steps of the cycle. The core of the cell-cycle control system is a series of molecular switches that operate in a defined sequence and orchestrate the main events of the cycle, including DNA replication and the segregation of duplicated chromosomes. In this section, we review the protein components of the control system and discuss how they work together to trigger the different phases of the cycle.

### The Cell-Cycle Control System Depends on Cyclically Activated Protein Kinases Called Cdks

The cell-cycle control system governs the cell-cycle machinery by cyclically activating and then inactivating the key proteins and protein complexes that initiate or regulate DNA replication, mitosis, and cytokinesis. This regulation is carried out largely through the phosphorylation and dephosphorylation of proteins involved in these essential processes.

As discussed in Chapter 4, phosphorylation followed by dephosphorylation is one of the most common ways by which cells switch the activity of a protein on and off (see Figure 4–46), and the cell-cycle control system

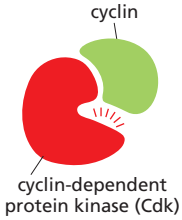



**Figure 18–4 Progression through the cell cycle depends on cyclin-dependent protein kinases (Cdks).** A Cdk must bind a regulatory protein called a cyclin before it can become enzymatically active. This activation also requires an activating phosphorylation of the Cdk (not shown, but see **Movie 18.1**). Once activated, a cyclin–Cdk complex phosphorylates key proteins in the cell that are required to initiate particular steps in the cell cycle. The cyclin also helps direct the Cdk to the target proteins that the Cdk phosphorylates.

uses this mechanism extensively and repeatedly. The phosphorylation reactions that control the cell cycle are carried out by a specific set of protein kinases, while dephosphorylation is performed by a set of protein phosphatases.

The protein kinases at the core of the cell-cycle control system are present in proliferating cells throughout the cell cycle. They are activated, however, only at appropriate times in the cycle, after which they are quickly inactivated. Thus, the activity of each of these kinases rises and falls in a cyclical fashion. Some of these protein kinases, for example, become active toward the end of $G_1$ phase and are responsible for driving the cell into S phase; another kinase becomes active just before M phase and drives the cell into mitosis.

Switching these kinases on and off at the appropriate times is partly the responsibility of another set of proteins in the control system—the **cyclins**. Cyclins have no enzymatic activity themselves, but they must bind to the cell-cycle kinases before the kinases can become enzymatically active. The kinases of the cell-cycle control system are therefore known as **cyclin-dependent protein kinases**, or **Cdks** (**Figure 18–4**). Cyclins are so-named because, unlike the Cdks, their concentrations vary in a cyclical fashion during the cell cycle. The cyclical changes in cyclin concentrations help drive the cyclic assembly and activation of the cyclin–Cdk complexes. Once activated, cyclin–Cdk complexes help trigger various cell-cycle events, such as entry into S phase or M phase (**Figure 18–5**). We discuss how the Cdks and cyclins were discovered in **How We Know**, pp. 615–616.

## Different Cyclin–Cdk Complexes Trigger Different Steps in the Cell Cycle

There are several types of cyclins and, in most eukaryotes, several types of Cdks involved in cell-cycle control. Different cyclin–Cdk complexes trigger different steps of the cell cycle. As shown in Figure 18–5, the cyclin that acts in $G_2$ to trigger entry into M phase is called **M cyclin**, and the active complex it forms with its Cdk is called **M-Cdk**. Other cyclins, called **S cyclins** and **$G_1$/S cyclins**, bind to a distinct Cdk protein late in $G_1$ to form **S-Cdk** and **$G_1$/S-Cdk**, respectively; these cyclin–Cdk complexes help launch S phase. The rise and fall of S cyclin and M cyclin concentrations

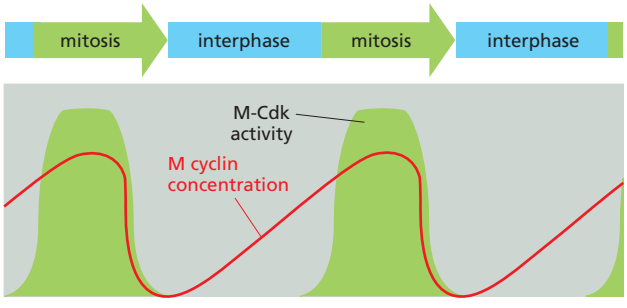



**Figure 18–5 The accumulation of cyclins helps regulate the activity of Cdks.** The formation of active cyclin–Cdk complexes drives various cell-cycle events, including entry into S phase or M phase. The figure shows the changes in cyclin concentration and Cdk protein kinase activity responsible for controlling entry into M phase. Increasing concentration of the relevant cyclin (called M cyclin) helps direct the formation of the active cyclin–Cdk complex (M-Cdk) that drives entry into M phase. Although the enzymatic activity of each type of cyclin–Cdk complex rises and falls during the course of the cell cycle, the concentration of the Cdk component does not (not shown).

# DISCOVERY OF CYCLINS AND Cdks

For many years, cell biologists watched the "puppet show" of DNA synthesis, mitosis, and cytokinesis but had no idea what was behind the curtain, controlling these events. The cell-cycle control system was simply a "black box" inside the cell. It was not even clear whether there was a separate control system, or whether the cell-cycle machinery somehow controlled itself. A breakthrough came with the identification of the key proteins of the control system and the realization that they are distinct from the components of the cell-cycle machinery—the enzymes and other proteins that perform the essential processes of DNA replication, chromosome segregation, and so on.

The first components of the cell-cycle control system to be discovered were the cyclins and cyclin-dependent protein kinases (Cdks) that drive cells into M phase. They were found in studies of cell division conducted on animal eggs.

## Back to the egg

The fertilized eggs of many animals are especially suitable for biochemical studies of the cell cycle because they are exceptionally large and divide rapidly. An egg of the frog *Xenopus*, for example, is just over 1 mm in diameter (**Figure 18–6**). After fertilization, it divides rapidly to partition the egg into many smaller cells. These rapid cell cycles consist mainly of repeated S and M phases, with very short or no $G_1$ or $G_2$ phases between them. There is no new gene transcription: all of the mRNAs and most of the proteins required for this early stage of embryonic development are already packed into the very large egg during its development as an oocyte in the ovary of the mother. In these early division cycles (*cleavage divisions*), no cell growth occurs, and all the cells of the embryo divide synchronously, growing smaller and smaller with each division (**Movie 18.2**).

Because of the synchrony, it is possible to prepare an extract from frog eggs that is representative of the cell-cycle stage at which the extract is made. The biological activity of such an extract can then be tested by injecting it into a *Xenopus* oocyte (the immature precursor of the unfertilized egg) and observing, microscopically, its effects on cell-cycle behavior. The *Xenopus* oocyte is an especially convenient test system for detecting an activity that drives cells into M phase, because of its large size, and because it has completed DNA replication and is suspended at a stage in the meiotic cell cycle (discussed in Chapter 19) that is equivalent to the $G_2$ phase of a mitotic cell cycle.

## Give us an M

In such experiments, Kazuo Matsui and colleagues found that an extract from an M-phase egg instantly drives the oocyte into M phase, whereas cytoplasm from a cleaving egg at other phases of the cycle does not. When they first made this discovery, they did not know the molecules or the mechanism responsible, so they referred to the unidentified agent as *maturation promoting factor*, or MPF (**Figure 18–7**). By testing cytoplasm from different stages of the cell cycle, Matsui and colleagues found that MPF activity oscillates dramatically during the course of each cell cycle: it increased rapidly just before the start of mitosis and fell rapidly to zero toward the end of mitosis (see Figure 18–5). This oscillation made MPF a strong candidate for a component involved in cell-cycle control.

When MPF was finally purified, it was found to contain a protein kinase that was required for its activity. But the kinase portion of MPF did not act alone. It had to have a specific protein (now known to be M cyclin) bound to it in order to function. M cyclin was discovered in a different type of experiment, involving clam eggs.


**Figure 18–6 A mature *Xenopus* egg provides a convenient system for studying the cell cycle.** (Courtesy of Tony Mills.)

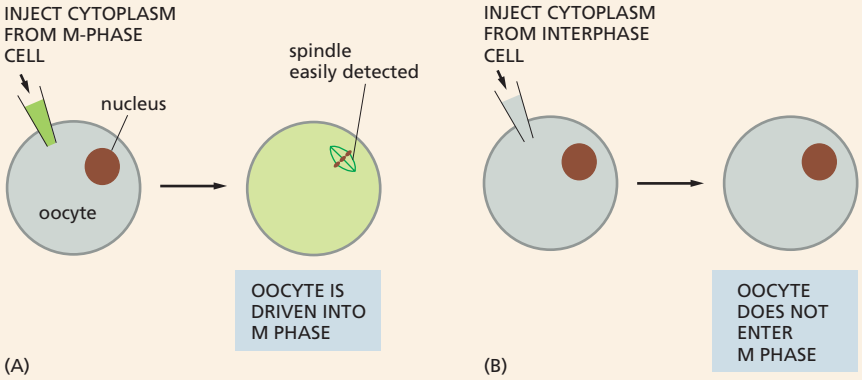



**Figure 18–7 MPF activity was discovered by injecting *Xenopus* egg cytoplasm into *Xenopus* oocytes.** (A) A *Xenopus* oocyte is injected with cytoplasm taken from a *Xenopus* egg in M phase. The cell extract drives the oocyte into M phase of the first meiotic division (a process called maturation), causing the large nucleus to break down and a spindle to form. (B) When the cytoplasm is instead taken from a cleaving egg in interphase, it does not cause the oocyte to enter M phase. Thus, the extract in (A) must contain some activity—a maturation promoting factor (MPF)—that triggers entry into M phase.

## Fishing in clams

M cyclin was initially identified by Tim Hunt as a protein whose concentration rose gradually during interphase and then fell rapidly to zero as cleaving clam eggs went through M phase (see Figure 18–5). The protein repeated this performance in each cell cycle. Its role in cell-cycle control, however, was initially obscure. The breakthrough occurred when cyclin was found to be a component of MPF and to be required for MPF activity. Thus, MPF, which we now call M-Cdk, is a protein complex containing two subunits—a regulatory subunit, M cyclin, and a catalytic subunit, the mitotic Cdk. After the components of M-Cdk were identified, other types of cyclins and Cdks were isolated, whose concentrations or activities, respectively, rose and fell at other stages in the cell cycle.

## All in the family

While biochemists were identifying the proteins that regulate the cell cycles of frog and clam embryos, yeast geneticists—led by Lee Hartwell, studying baker's yeast (*Saccharomyces cerevisiae*), and Paul Nurse, studying fission yeast (*S. pombe*)—were taking a genetic approach to dissecting the cell-cycle control system. By studying mutants that get stuck or misbehave at specific points in the cell cycle, these researchers were able to identify many genes responsible for cell-cycle control. Some of these genes turned out to encode cyclin or Cdk proteins, which were unmistakably similar—in both amino acid sequence and function—to their counterparts in frogs and clams. Similar genes were soon identified in human cells.

Many of the cell-cycle control genes have changed so little during evolution that the human version of the gene will function perfectly well in a yeast cell. For example, Nurse and colleagues were the first to show that a yeast with a defective copy of the gene encoding its only Cdk fails to divide, but it divides normally if a copy of the appropriate human gene is artificially introduced into the defective cell. Surely, even Darwin would have been astonished at such clear evidence that humans and yeasts are cousins. Despite a billion years of divergent evolution, all eukaryotic cells—whether yeast, animal, or plant—use essentially the same molecules to control the events of their cell cycle.

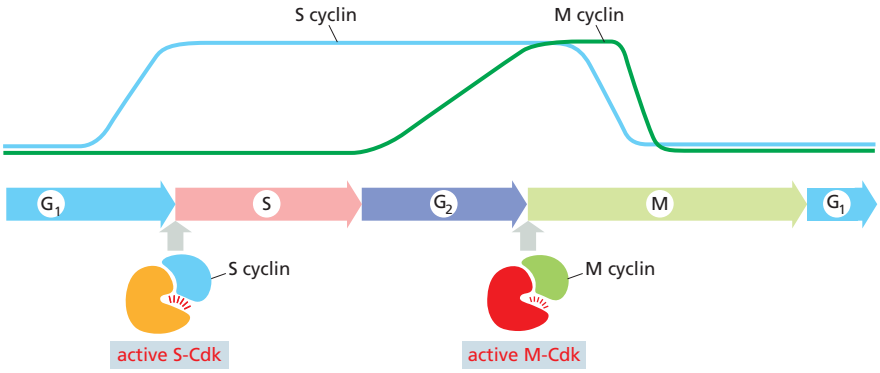



**Figure 18–8 Distinct Cdks associate with different cyclins to trigger the different events of the cell cycle.** For simplicity, only two types of cyclin–Cdk complexes are shown: one that triggers S phase and one that triggers M phase.

are shown in Figure 18–8. Another group of cyclins, called **$G_1$ cyclins**, act earlier in $G_1$ and bind to other Cdk proteins to form **$G_1$-Cdks**, which help drive the cell through $G_1$ toward S phase. We see later that the formation of these $G_1$-Cdks in animal cells usually depends on extracellular signal molecules that stimulate cells to divide. The names of the main cyclins and their Cdks are listed in Table 18–2.

Each of these cyclin–Cdk complexes phosphorylates a different set of target proteins in the cell. $G_1$/S-Cdks, for example, phosphorylate regulatory proteins that activate transcription of genes required for DNA replication. By activating different sets of target proteins, each type of complex triggers a different transition step in the cell cycle.

## Cyclin Concentrations Are Regulated by Transcription and by Proteolysis

As discussed in Chapter 7, the concentration of a given protein in the cell is determined by the rate at which the protein is synthesized and the rate at which it is degraded. Over the course of the cell cycle, the concentration of each type of cyclin rises gradually and then falls abruptly (see Figure 18–8). The gradual increase in cyclin concentration stems from continued transcription of cyclin genes and synthesis of cyclin proteins, whereas the rapid fall in cyclin concentration is precipitated by a full-scale targeted destruction of the protein.

The abrupt degradation of M and S cyclins partway through M phase depends on a large enzyme called—for reasons that will become clear later—the **anaphase-promoting complex** or cyclosome (**APC/C**). This complex tags these cyclins with a chain of ubiquitin. As discussed in Chapter 7, proteins marked in this way are directed to proteasomes where they are rapidly degraded (see Figure 7–43). The ubiquitylation and degradation of the cyclin returns its Cdk to an inactive state (Figure 18–9).

**TABLE 18–2 THE MAJOR CYCLINS AND CDKS OF VERTEBRATES**

| Cyclin–Cdk Complex | Cyclin | Cdk Partner |
|---|---|---|
| $G_1$-Cdk | cyclin D* | Cdk4, Cdk6 |
| $G_1$/S-Cdk | cyclin E | Cdk2 |
| S-Cdk | cyclin A | Cdk2 |
| M-Cdk | cyclin B | Cdk1 |

*There are three forms of cyclin D in mammals (cyclins D1, D2, and D3).

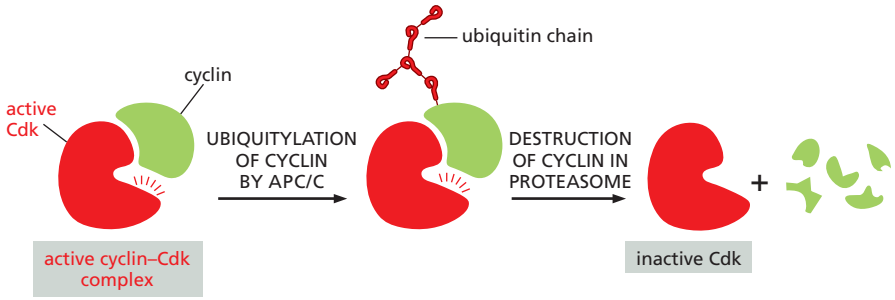



**Figure 18–9 The activity of some Cdks is regulated by cyclin degradation.** Ubiquitylation of S or M cyclin by APC/C marks the protein for destruction in proteasomes (as discussed in Chapter 7). The loss of cyclin renders its Cdk partner inactive.

Like cyclin accumulation, cyclin destruction can also help drive the transition from one phase of the cell cycle to the next. For example, M cyclin degradation—and the resulting inactivation of M-Cdk—leads to the molecular events that take the cell out of mitosis.

## The Activity of Cyclin–Cdk Complexes Depends on Phosphorylation and Dephosphorylation

The appearance and disappearance of cyclin proteins play an important part in regulating Cdk activity during the cell cycle, but there is more to the story: although cyclin concentrations increase gradually, the activity of the associated cyclin–Cdk complexes tends to switch on abruptly at the appropriate time in the cell cycle (see Figure 18–5). What triggers the abrupt activation of these complexes? It turns out that the cyclin–Cdk complex contains inhibitory phosphates, and to become active, the Cdk must be dephosphorylated by a specific protein phosphatase (**Figure 18–10**). Thus protein kinases and phosphatases act together to regulate the activity of specific cyclin–Cdk complexes and help control progression through the cell cycle.

## Cdk Activity Can Be Blocked by Cdk Inhibitor Proteins

In addition to phosphorylation and dephosphorylation, the activity of Cdks can also be modulated by the binding of **Cdk inhibitor proteins**. The cell-cycle control system uses these inhibitors to block the assembly or activity of certain cyclin–Cdk complexes. Some Cdk inhibitor proteins, for example, help maintain Cdks in an inactive state during the $G_1$ phase of the cycle, thus delaying progression into S phase (**Figure 18–11**). Pausing at this transition point in $G_1$ gives the cell more time to grow, or allows it to wait until extracellular conditions are favorable for division.

## The Cell-Cycle Control System Can Pause the Cycle in Various Ways

As mentioned earlier, the cell-cycle control system can transiently delay progress through the cycle at various transition points to ensure that the major events of the cycle occur only when the cell is fully prepared (see

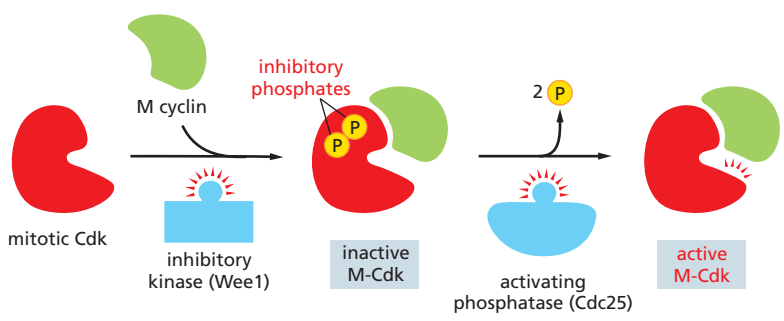



**Figure 18–10 For M-Cdk to be active, inhibitory phosphates must be removed.** As soon as the M cyclin–Cdk complex is formed, it is phosphorylated at two adjacent sites by an inhibitory protein kinase called Wee1. This modification keeps M-Cdk in an inactive state until these phosphates are removed by an activating protein phosphatase called Cdc25. It is still not clear how the timing of the critical Cdc25 phosphatase triggering step shown here is controlled.

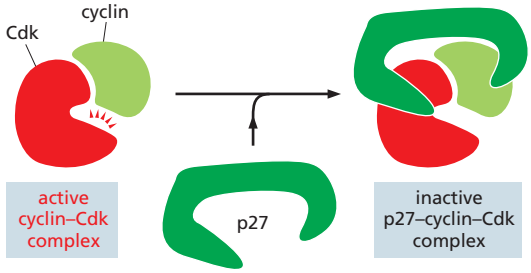



**Figure 18–11 The activity of a Cdk can be blocked by the binding of a Cdk inhibitor.** In this instance, the inhibitor protein (called p27) binds to an activated cyclin–Cdk complex. Its attachment prevents the Cdk from phosphorylating target proteins required for progress through $G_1$ into S phase.

Figure 18–3). At these transitions, the control system monitors the cell's internal state and the conditions in its environment, before allowing the cell to continue through the cycle. For example, it allows entry into S phase only if environmental conditions are appropriate; it triggers mitosis only after the DNA has been completely replicated; and it initiates chromosome segregation only after the duplicated chromosomes are correctly aligned on the mitotic spindle.

To accomplish these feats, the control system uses a combination of the mechanisms we have described. At the $G_1$-to-S transition, it uses Cdk inhibitors to keep cells from entering S phase and replicating their DNA (see Figure 18–11). At the $G_2$-to-M transition, it suppresses the activation of M-Cdk by inhibiting the phosphatase required to activate the Cdk (see Figure 18–10). And it can delay the exit from mitosis by inhibiting the activation of APC/C, thus preventing the degradation of M cyclin (see Figure 18–9).

These mechanisms, summarized in **Figure 18–12**, allow the cell to make "decisions" about whether to progress through the cell cycle or to arrest in the current phase and await more favorable conditions. In the next section, we take a closer look at how the cell-cycle control system decides whether a cell in $G_1$ should commit to divide.

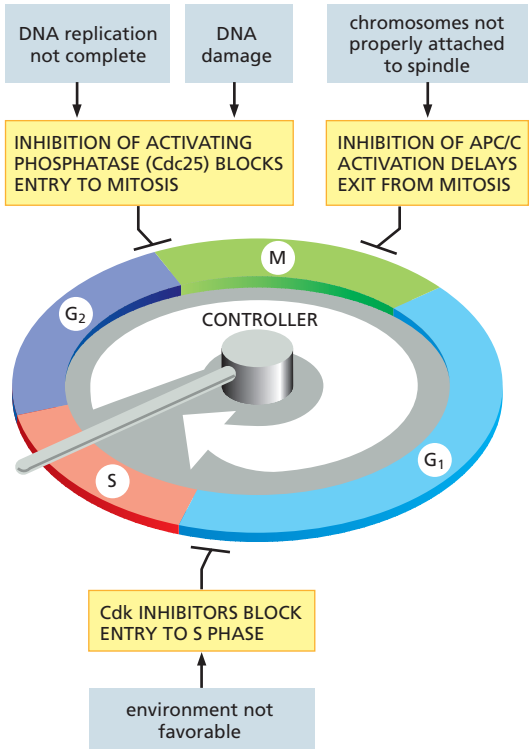



**Figure 18–12 The cell-cycle control system uses various mechanisms to pause the cycle at specific transition points.**

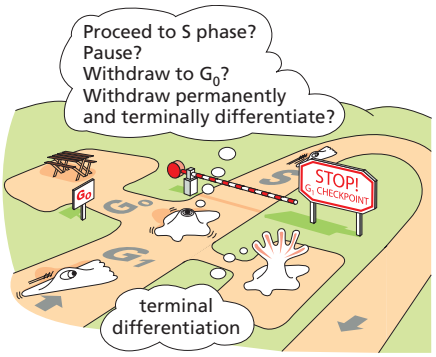



**Figure 18–13 The transition from $G_1$ to S phase offers the cell a crossroad.** The cell can commit to completing another cell cycle, pause temporarily until conditions are right, or withdraw from the cell cycle altogether—either temporarily in $G_0$, or permanently in the case of terminally differentiated cells.

## QUESTION 18–3

Why do you suppose cells have evolved a special $G_0$ phase to exit from the cell cycle, rather than just stopping in $G_1$ and not moving on to S phase?

# $G_1$ PHASE

In addition to being a bustling period of metabolic activity, cell growth, and repair, $G_1$ serves as an important time of decision-making for the cell. Based on intracellular signals that provide information about the size of the cell and extracellular signals reflecting conditions in the environment, the cell-cycle control machinery can either hold the cell transiently in $G_1$ (or in a more prolonged nonproliferative state, $G_0$), or allow it to prepare for entry into the S phase of another cell cycle. Once past this critical $G_1$-to-S transition, a cell usually continues all the way through the rest of the cell cycle. In yeasts, the $G_1$-to-S transition is therefore sometimes called Start, because passing it represents a commitment to complete a full cell cycle (**Figure 18–13**).

In this section, we consider how the cell-cycle control system decides whether to proceed to S phase and commit to another cell cycle—and what happens once the decision is made. The molecular mechanisms involved are especially important, as defects in them can lead to unrestrained cell proliferation and cancer.

## Cdks Are Stably Inactivated in $G_1$

During early M phase, when mitosis begins, the cell is awash with active cyclin–Cdk complexes. Those S-Cdks and M-Cdks must be disabled by the end of M phase to allow the cell to complete division and to prevent it from initiating another round of division without spending any time in $G_1$.

To usher a cell from the upheaval of M phase to the relative tranquility of $G_1$, the cell-cycle control machinery must inactivate its inventory of S-Cdk and M-Cdk. It does so in several ways: by eliminating all of the existing cyclins, by blocking the synthesis of new ones, and by deploying Cdk inhibitor proteins to muffle the activity of any remaining cyclin–Cdk complexes. The use of multiple mechanisms makes this system of suppression robust, ensuring that essentially all Cdk activity is shut down. This wholesale inactivation resets the cell-cycle control system and generates a stable $G_1$ phase, during which the cell can grow and monitor its environment before committing to a new round of division.

## Mitogens Promote the Production of the Cyclins That Stimulate Cell Division

As a general rule, mammalian cells will multiply only if they are stimulated to do so by extracellular signals, called *mitogens*, produced by other cells. If deprived of such signals, the cell cycle arrests in $G_1$; if the cell is deprived of mitogens for long enough, it will withdraw from the cell cycle and enter a nonproliferating state, in which the cell can remain for days or weeks, months, or even for the lifetime of the organism, as we discuss shortly.

Escape from cell-cycle arrest—or from certain nonproliferating states—requires the accumulation of cyclins. Mitogens act by switching on cell signaling pathways that stimulate the synthesis of $G_1$ cyclins, $G_1$/S cyclins, and other proteins involved in DNA synthesis and chromosome duplication. The buildup of these cyclins triggers a wave of $G_1$/S-Cdk activity, which ultimately relieves the negative controls that otherwise block progression from $G_1$ to S phase.

One crucial negative control is provided by the *Retinoblastoma* (*Rb*) *protein*. Rb was initially identified from studies of a rare childhood eye tumor called retinoblastoma, in which the Rb protein is missing or defective.

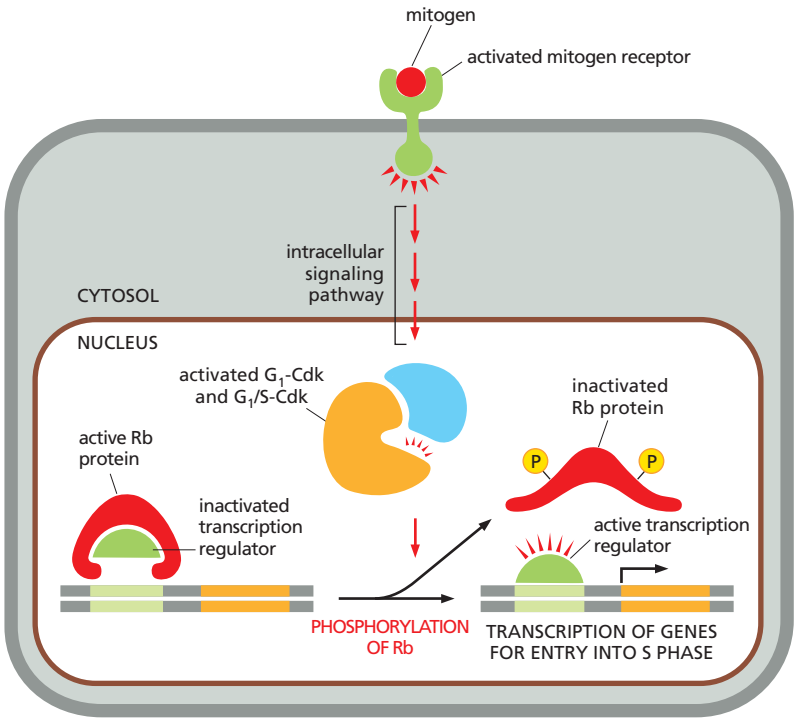



**Figure 18–14 One way in which mitogens stimulate cell proliferation is by inhibiting the Rb protein.** In the absence of mitogens, dephosphorylated Rb protein holds specific transcription regulators in an inactive state. Mitogens binding to cell-surface receptors activate intracellular signaling pathways that lead to the formation and activation of $G_1$-Cdk and $G_1$/S-Cdk complexes. These complexes phosphorylate, and thereby inactivate, the Rb protein, releasing the transcription regulators needed to activate the transcription of genes required for entry into S phase.

Rb is abundant in the nuclei of all vertebrate cells, where it binds to particular transcription regulators and prevents them from turning on the genes required for cell proliferation. Mitogens release the Rb brake by triggering the activation of $G_1$-Cdks and $G_1$/S-Cdks. These complexes phosphorylate the Rb protein, altering its conformation so that it releases its bound transcription regulators, which are then free to activate the genes required for entry into S phase (**Figure 18–14**).

## DNA Damage Can Temporarily Halt Progression Through $G_1$

The cell-cycle control system uses several distinct mechanisms to halt progress through the cell cycle if DNA is damaged, and it can do so at various transition points. The mechanism that operates at the $G_1$-to-S transition, which prevents the cell from replicating damaged DNA, is especially well understood. DNA damage in $G_1$ causes an increase in both the concentration and activity of a protein called **p53**, which is a transcription regulator that activates the gene encoding a Cdk inhibitor protein called p21. The p21 protein binds to $G_1$/S-Cdk and S-Cdk, preventing them from driving the cell into S phase (**Figure 18–15**). The arrest of the cell cycle in $G_1$ gives the cell time to repair the damaged DNA before replicating it. If the DNA damage is too severe to be repaired, p53 can induce the cell to kill itself through *apoptosis*, a form of programmed cell death we discuss later. If p53 is missing or defective, the unrestrained replication of damaged DNA leads to a high rate of mutation and the generation of cells that tend to become cancerous. In fact, mutations in the *p53* gene are found in about half of all human cancers (**Movie 18.3**).

## Cells Can Delay Division for Prolonged Periods by Entering Specialized Nondividing States

As mentioned earlier, cells can delay progress through the cell cycle at specific transition points, to wait for suitable conditions or to repair

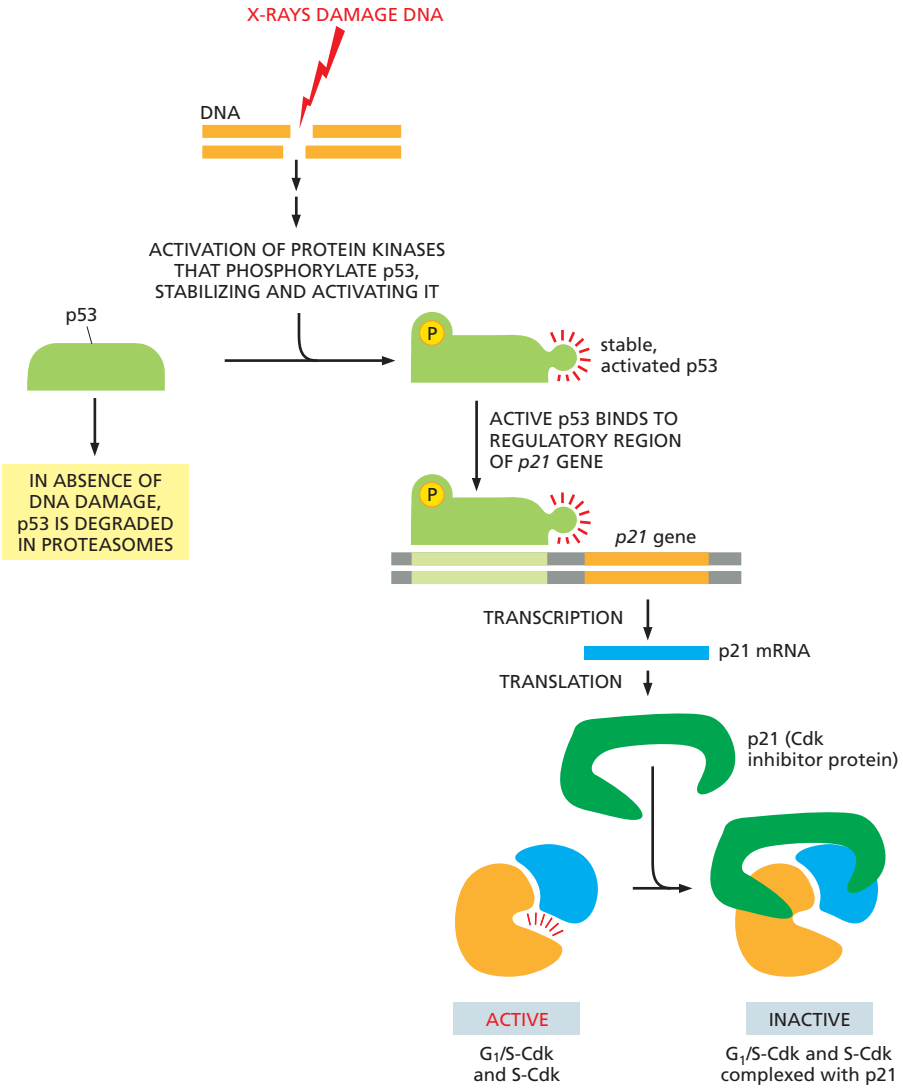



**Figure 18–15 DNA damage can arrest the cell cycle in $G_1$.** When DNA is damaged, specific protein kinases respond by both activating the p53 protein and halting its otherwise rapid degradation. Activated p53 protein thus accumulates and stimulates the transcription of the gene that encodes the Cdk inhibitor protein p21. The p21 protein binds to $G_1$/S-Cdk and S-Cdk and inactivates them, so that the cell cycle arrests in $G_1$.

damaged DNA. They can also withdraw from the cell cycle for prolonged periods—either temporarily or permanently.

The most radical decision that the cell-cycle control system can make is to withdraw the cell from the cell cycle permanently. This decision has a special importance in multicellular organisms. Many cells in the human body permanently stop dividing when they differentiate. In such *terminally differentiated* cells, such as nerve or muscle cells, the cell-cycle control system is dismantled completely and genes encoding the relevant cyclins and Cdks are irreversibly shut down.

In the absence of appropriate signals, other cell types withdraw from the cell cycle only temporarily, entering an arrested state called $G_0$. They retain the ability to reassemble the cell-cycle control system quickly and to divide again. Most liver cells, for example, are in $G_0$, but they can be stimulated to proliferate if the liver is damaged.

Much of the diversity in cell-division rates in the adult body lies in the variation in the time that cells spend in $G_0$ or in $G_1$. Some cell types, including liver cells, normally divide only once every year or two, whereas certain epithelial cells in the gut divide more than twice a day to renew the lining of the gut continually. Many of our cells fall somewhere in between: they can divide if the need arises but normally do so infrequently.

## QUESTION 18–4

What might be the consequences if a cell replicated damaged DNA before repairing it?

# S PHASE

Before a cell divides, it must replicate its DNA. As we discuss in Chapter 6, this replication must occur with extreme accuracy to minimize the risk of mutations in the next cell generation. Of equal importance, every nucleotide in the genome must be copied once—and only once—to prevent the damaging effects of gene amplification. In this section, we consider the elegant molecular mechanisms by which the cell-cycle control system initiates DNA replication and, at the same time, prevents replication from happening more than once per cell cycle.

## S-Cdk Initiates DNA Replication and Blocks Re-Replication

Like any monumental task, configuring chromosomes for replication requires a certain amount of preparation. For eukaryotic cells, this preparation begins early in $G_1$, when DNA is made replication-ready by the recruitment of proteins to the sites along each chromosome where replication will begin. These nucleotide sequences, called *origins of replication*, serve as landing pads for the proteins and protein complexes that control and carry out DNA synthesis, as discussed in Chapter 6.

One of these protein complexes, called the *origin recognition complex* (*ORC*), remains perched on the replication origins throughout the cell cycle. To prepare the DNA for replication, the ORC recruits a protein called Cdc6, whose concentration rises early in $G_1$. Together, these proteins load the DNA helicases that will ultimately open up the double helix at the origin of replication. Once this *prereplicative complex* is in place, the replication origin is loaded and ready to "fire."

The signal to commence replication comes from S-Cdk, the cyclin–Cdk complex that triggers S phase. S-Cdk is assembled and activated at the end of $G_1$. During S phase, S-Cdk activates the DNA helicases in the prereplicative complex and promotes the assembly of the rest of the proteins that form the *replication fork* (see Figure 6–20). In doing so, S-Cdk essentially "pulls the trigger" that initiates DNA replication (**Figure 18–16**).

In addition to triggering the initiation of DNA synthesis at a replication origin, S-Cdk also helps prevent re-replication. It does so by phosphorylating both Cdc6 and the ORC. Phosphorylation inactivates these proteins and helps prevent the reassembly of the prereplicative complex. These safeguards help ensure that DNA replication cannot be reinitiated later in the same cell cycle. When Cdks are inactivated in the next $G_1$ phase, the ORC and Cdc6 are reactivated, thereby allowing origins to be prepared for the following S phase.

## Incomplete Replication Can Arrest the Cell Cycle in $G_2$

Earlier, we described how DNA damage can signal the cell-cycle control system to delay progress through the $G_1$-to-S transition, preventing the cell from replicating damaged DNA. But what if errors occur during DNA replication—or if replication is delayed? How does the cell keep from dividing with DNA that is incorrectly or incompletely replicated?

To address these issues, the cell-cycle control system uses a mechanism that can delay entry into M phase. As we saw in Figure 18–10, the activity of M-Cdk is inhibited by phosphorylation at particular sites. For the cell to progress into mitosis, these inhibitory phosphates must be removed by an activating protein phosphatase called Cdc25. If DNA replication stalls, the appearance of single-stranded DNA at the replication fork triggers a DNA damage response. Part of this response includes the inhibition of the phosphatase Cdc25, which prevents the removal of the inhibitory

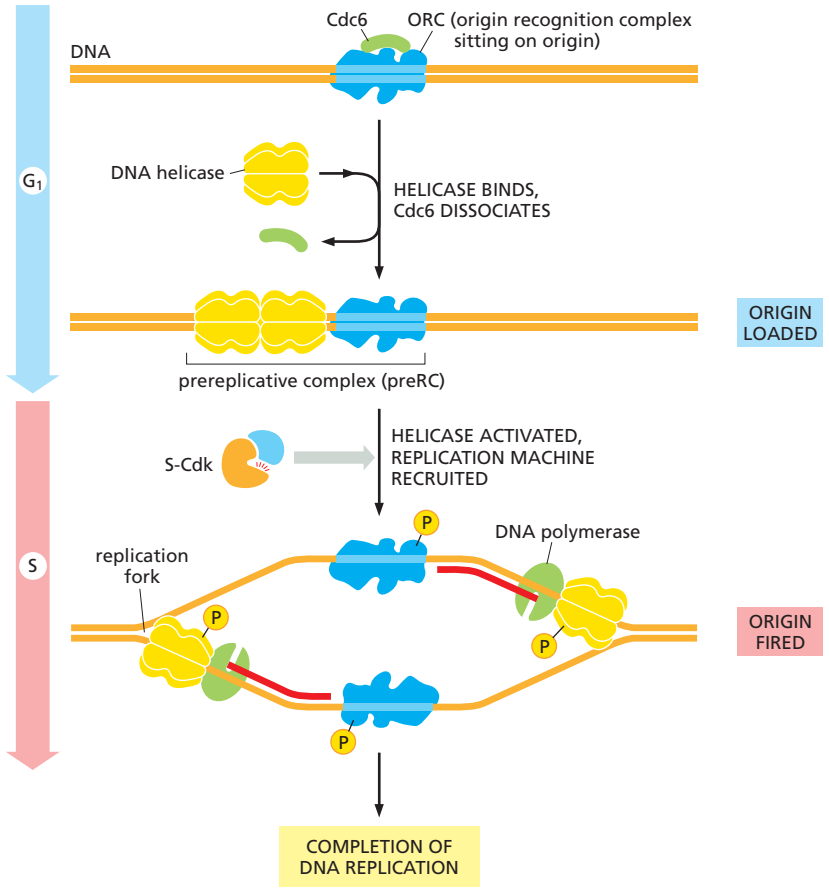



**Figure 18–16 The initiation of DNA replication takes place in two steps.** During $G_1$, Cdc6 binds to the ORC, and together these proteins load a pair of DNA helicases on the DNA to form the prereplicative complex. At the start of S phase, S-Cdk triggers the firing of this loaded replication origin by guiding the assembly of the DNA polymerase (*green*) and other proteins (not shown) that initiate DNA synthesis at the replication fork (discussed in Chapter 6). S-Cdk also blocks re-replication by phosphorylating Cdc6 (not shown) and the ORC. This phosphorylation keeps these proteins inactive and prevents the reassembly of the prereplicative complex until the Cdks are turned off in the next $G_1$.

phosphates from M-Cdk. As a result, M-Cdk remains inactive and M phase is delayed until DNA replication is complete and any DNA damage is repaired.

Once a cell has successfully replicated its DNA in S phase, and progressed through $G_2$, it is ready to enter M phase. During this relatively brief period, the cell will accomplish a remarkable reconfiguration, dividing its nucleus (mitosis) and then its cytoplasm (cytokinesis; see Figure 18–2). In the next three sections, we describe the events that occur during M phase. We first present a brief overview of M phase as a whole and then discuss, in sequence, the mechanics of mitosis and of cytokinesis, with a focus on animal cells.

## M PHASE

Although M phase (which includes mitosis plus cytokinesis) takes place over a relatively short amount of time—about one hour in a mammalian cell—it is by far the most dramatic phase of the cell cycle. During this brief period, the cell reorganizes virtually all of its components and distributes them equally into the two daughter cells. The earlier phases of the cell cycle, in effect, set the stage for the drama of M phase.

The central problem for a cell in M phase is to accurately segregate the chromosomes that were duplicated in the preceding S phase, so that each new daughter cell receives an identical copy of the genome. With minor variations, all eukaryotes solve this problem in a similar way: they assemble two specialized cytoskeletal machines—one that pulls the duplicated chromosomes apart (during mitosis) and another that divides the cytoplasm into two halves (during cytokinesis). We begin our discussion of M phase with an overview of how the cell sets the processes of M phase in motion.

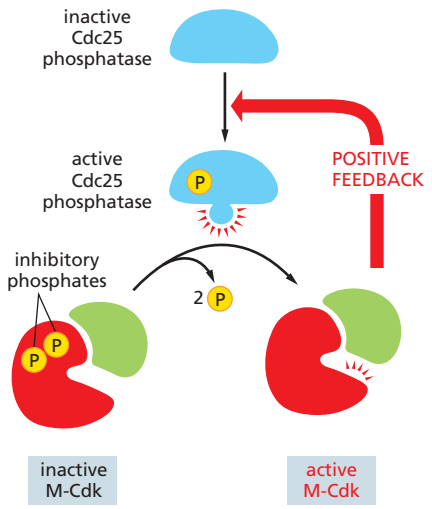



**Figure 18–17 Activated M-Cdk indirectly activates more M-Cdk, creating a positive feedback loop.** Once activated, M-Cdk phosphorylates, and thereby activates, more Cdk-activating phosphatase (Cdc25). This phosphatase can now activate more M-Cdk by removing the inhibitory phosphate groups from the Cdk subunit.

## M-Cdk Drives Entry into Mitosis

One of the most remarkable features of the cell-cycle control system is that a single protein complex, M-Cdk, brings about all the diverse and intricate rearrangements that occur in the early stages of mitosis. Among its many duties, M-Cdk helps prepare the duplicated chromosomes for segregation and induces the assembly of the mitotic spindle—the machinery that will pull the duplicated chromosomes apart.

M-Cdk complexes accumulate throughout $G_2$. But this stockpile is not switched on until the end of $G_2$, when the activating phosphatase Cdc25 removes the inhibitory phosphates holding M-Cdk activity in check. This act of activation is self-reinforcing: once activated, each M-Cdk complex can indirectly turn on additional M-Cdk complexes—by phosphorylating and activating more Cdc25 (**Figure 18–17**). Activated M-Cdk also shuts down the inhibitory kinase Wee1 (see Figure 18–10), further promoting the production of activated M-Cdk. The overall consequence is that, once M-Cdk activation begins, it ignites an explosive increase in M-Cdk activity that drives the cell abruptly—and irreversibly—from $G_2$ into M phase.

The same M-Cdk complexes that drive entry into mitosis also help set the stage for its exit. Activated M-Cdk turns on APC/C, which—after a period of delay—directs the destruction of M cyclin and, ultimately, the inactivation of M-Cdk.

## Cohesins and Condensins Help Configure Duplicated Chromosomes for Separation

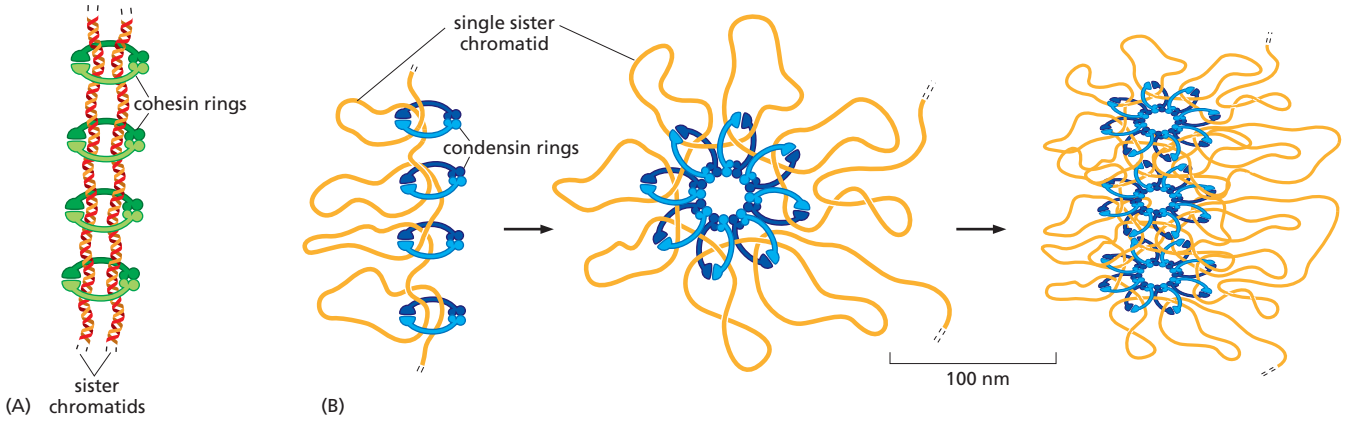
To ensure that duplicated chromosomes will be properly separated during mitosis, two related protein complexes help cells manage and keep track of the replicated DNA. The first complexes come into play during S phase. When a chromosome is duplicated, the two copies remain tightly bound together. These identical copies—called **sister chromatids**—each contain a single, double-stranded molecule of DNA, along with its associated proteins. The sisters are held together by protein complexes called **cohesins**, which assemble along the length of each chromatid as the DNA is replicated. This cohesion between sister chromatids is crucial for proper chromosome segregation, and it is broken completely only in late mitosis to allow the sisters to be pulled apart by the mitotic spindle. Defects in sister-chromatid cohesion lead to major errors in chromosome segregation. In humans, such mis-segregation can lead to abnormal numbers of chromosomes, resulting in genetic imbalances that are usually deleterious or even lethal.

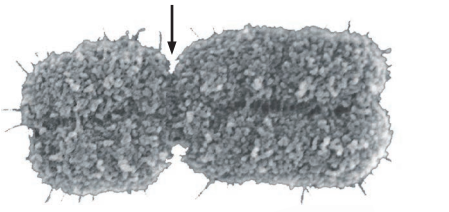
When the cell enters M phase, the duplicated chromosomes condense, becoming visible under the microscope. Protein complexes called **condensins** help carry out this **chromosome condensation**, which reduces mitotic chromosomes to compact bodies that can be more easily segregated within the crowded confines of the dividing cell. The assembly of condensin complexes onto the DNA is triggered by the phosphorylation of condensins by M-Cdk.

Cohesins and condensins are structurally related, and both are thought to form ring structures around chromosomal DNA. However, whereas cohesins encircle the two sister chromatids, tying them together (**Figure 18–18A**), condensins assemble along each individual sister chromatid,

## QUESTION 18–5

A small amount of cytoplasm isolated from a mitotic cell is injected into an unfertilized frog oocyte, causing the oocyte to enter M phase (see Figure 18–7A). A sample of the injected oocyte's cytoplasm is then taken and injected into a second oocyte, causing this cell also to enter M phase. The process is repeated many times until, essentially, none of the original protein sample remains, and yet, cytoplasm taken from the last in the series of injected oocytes is still able to trigger entry into M phase with undiminished efficiency. Explain this remarkable observation.



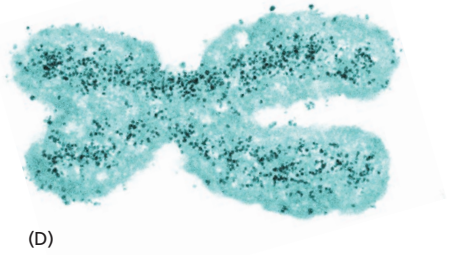




**Figure 18–18 Cohesins and condensins help to configure duplicated chromosomes for segregation.** (A) Cohesins tie together the two adjacent sister chromatids in each duplicated chromosome. They are thought to form large protein rings that surround the sister chromatids, preventing them from coming apart, until the rings are broken late in mitosis. (B) Condensins help coil each sister chromatid (in other words, each DNA double helix) into a smaller, more compact structure that can be more easily segregated during mitosis. These cartoons illustrate one way that condensins might package chromatids; the exact mechanism is not known. (C) A scanning electron micrograph of a condensed, duplicated mitotic chromosome, consisting of two sister chromatids joined along their length. The constricted region (*arrow*) is the centromere, where each chromatid will attach to the mitotic spindle, which pulls the sister chromatids apart toward the end of mitosis. (D) An electron micrograph of a duplicated mitotic chromosome in which condensin is labelled with antibodies attached to tiny gold particles (*dark* dots), showing that condensins are found mainly in the central core of the chromosome. This centralized location of condensins is also represented in the cartoon model shown in (B). (C, courtesy of Terry D. Allen; D, adapted from N. Kireeva et al., *J. Cell Biol.* 166:775–785, 2004. With permission from Rockefeller University Press.)

helping each of these double helices to coil up into a more compact form (**Figure 18–18B–D**). Together, these proteins help configure replicated chromosomes for mitosis.

## Different Cytoskeletal Assemblies Carry Out Mitosis and Cytokinesis

After the duplicated chromosomes have condensed, a pair of complex cytoskeletal machines assemble in sequence to carry out the two mechanical processes that occur in M phase. The mitotic spindle carries out nuclear division (mitosis), and, in animal cells and many unicellular eukaryotes, the *contractile ring* carries out cytoplasmic division (cytokinesis) (**Figure 18–19**). Both structures disassemble rapidly after they have performed their tasks.

The mitotic spindle is composed of microtubules and the various proteins that interact with them, including microtubule-associated motor proteins (discussed in Chapter 17). In all eukaryotic cells, the mitotic spindle is

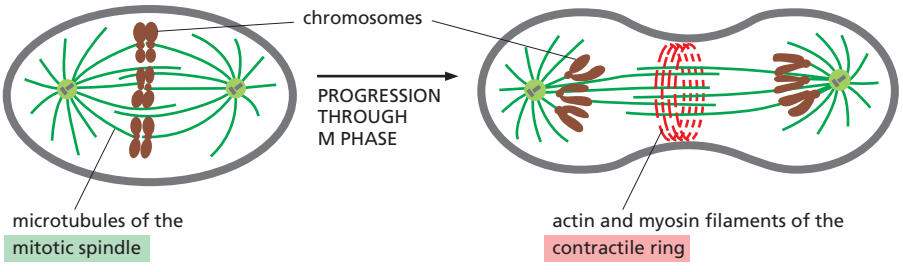



**Figure 18–19 Two transient cytoskeletal structures mediate M phase in animal cells.** The mitotic spindle assembles first to separate the duplicated chromosomes. Then, the contractile ring assembles to divide the cell in two. Whereas the mitotic spindle is based on microtubules, the contractile ring is based on actin and myosin. Plant cells use a very different mechanism to divide the cytoplasm, as we discuss later.

responsible for separating the duplicated chromosomes and allocating one copy of each chromosome to each daughter cell.

The *contractile ring* consists mainly of actin and myosin filaments arranged in a ring around the equator of the cell (see Chapter 17). It starts to assemble just beneath the plasma membrane toward the end of mitosis. As the ring contracts, it pulls the membrane inward, thereby dividing the cell in two (see Figure 18–19). We discuss later how plant cells, which have a cell wall to contend with, divide their cytoplasm by a very different mechanism.

### M Phase Occurs in Stages

Although M phase proceeds as a continuous sequence of events, it is traditionally divided into a series of stages. The first five stages of M phase—prophase, prometaphase, metaphase, anaphase, and telophase—constitute **mitosis**, which was originally defined as the period in which the chromosomes are visible in the microscope (because they have become condensed). *Cytokinesis*, which constitutes the final stage of M phase, begins before mitosis ends. The stages of M phase are summarized in **Panel 18–1** (pp. 628–629). Together, they form a dynamic sequence in which several independent cycles—involving the chromosomes, cytoskeleton, and centrosomes—are coordinated to produce two genetically identical daughter cells (**Movie 18.4** and **Movie 18.5**).

## MITOSIS

Before nuclear division, or mitosis, begins, each chromosome has been duplicated and consists of two identical sister chromatids, held together along their length by cohesin proteins (see Figure 18–18A). During mitosis, the cohesin proteins are removed, the sister chromatids split apart, and the chromosomes are pulled to opposite poles of the cell by the mitotic spindle (**Figure 18–20**). In this section, we examine how the mitotic spindle assembles and functions. We discuss how the dynamic instability of microtubules and the activity of microtubule-associated motor proteins contribute to both the assembly of the spindle and its ability to segregate the duplicated chromosomes. We then consider the mechanism that operates during mitosis to ensure the synchronous separation of these chromosomes. Finally, we discuss how the daughter nuclei form.

### Centrosomes Duplicate to Help Form the Two Poles of the Mitotic Spindle

Before M phase begins, two critical events must be completed: DNA must be fully replicated, and, in animal cells, the centrosome must be duplicated. The **centrosome** is the principal *microtubule-organizing center* in animal cells (see Figure 17–13). Duplication is necessary for the centrosome to be able to help form the two poles of the mitotic spindle and so that each daughter cell will receive its own centrosome.

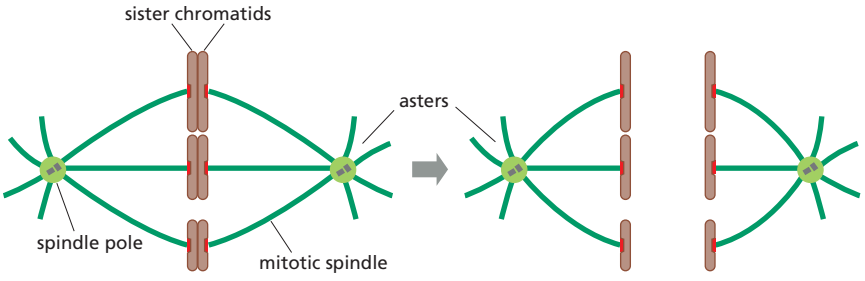
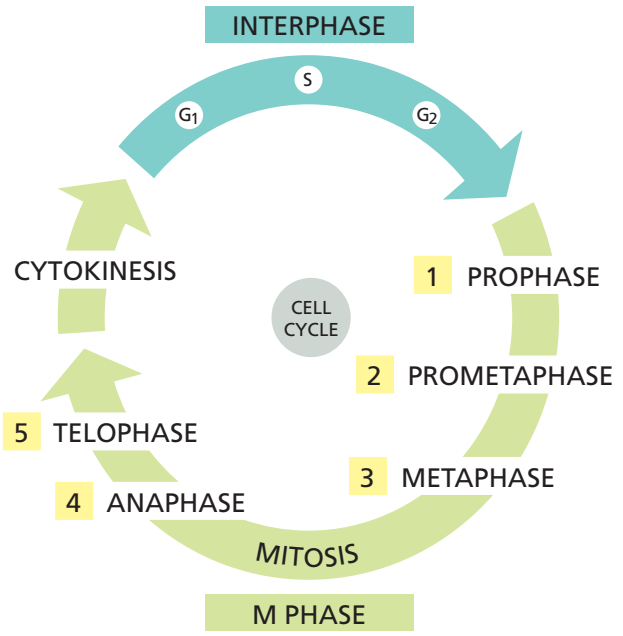



**Figure 18–20 Sister chromatids separate at the beginning of anaphase.** The mitotic spindle then pulls the separated sisters to opposite poles of the cell.

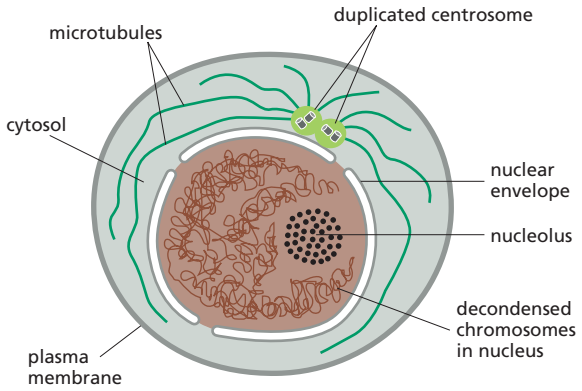
# PANEL 18–1 THE PRINCIPAL STAGES OF M PHASE IN AN ANIMAL CELL

## CELL DIVISION AND THE CELL CYCLE




The division of a cell into two daughters occurs in the M phase of the cell cycle. M phase consists of nuclear division, or mitosis, and cytoplasmic division, or cytokinesis. In this figure, M phase has been greatly expanded for clarity. Mitosis is itself divided into five stages, and these, together with cytokinesis, are described in this panel.

## INTERPHASE



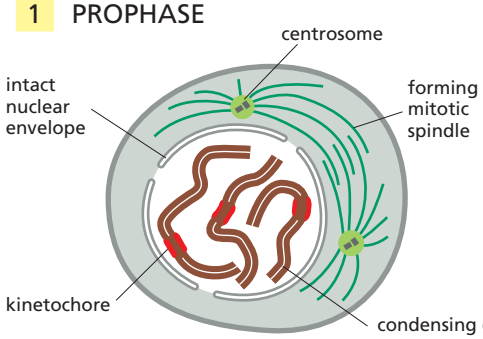

During interphase, the cell increases in size. The DNA of the chromosomes is replicated, and the centrosome is duplicated.

In the light micrographs of dividing animal cells shown in this panel, chromosomes are stained *orange* and microtubules are *green*.

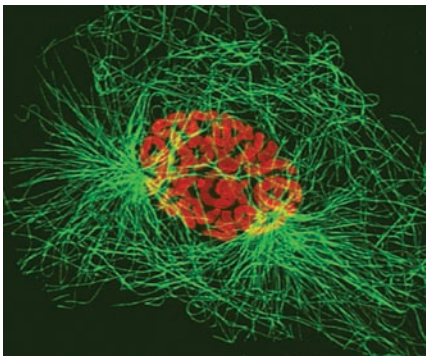
(Courtesy of Julie Canman and Ted Salmon.)

MITOSIS

## 1 PROPHASE

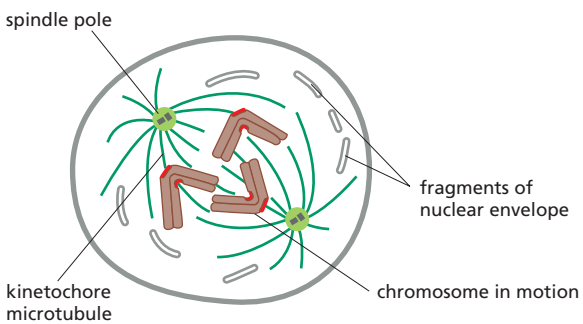



At prophase, the duplicated chromosomes, each consisting of two closely associated sister chromatids, condense. Outside the nucleus, the mitotic spindle assembles between the two centrosomes, which have begun to move apart. For simplicity, only three chromosomes are drawn.

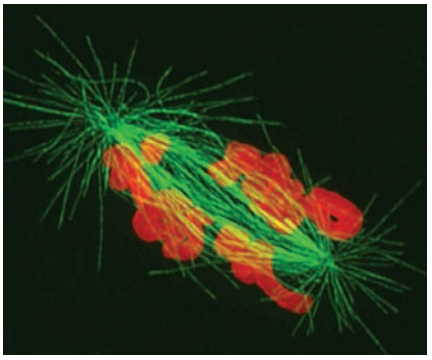


MITOSIS

## 2 PROMETAPHASE

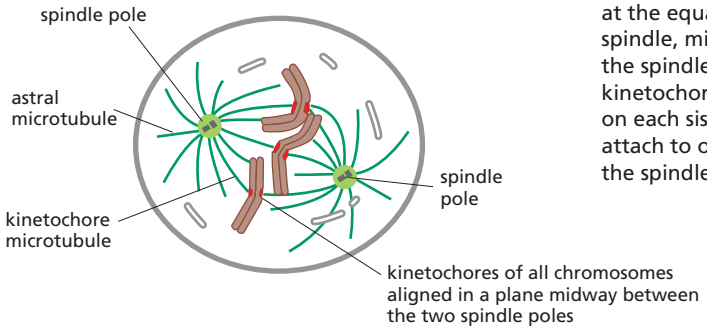



Prometaphase starts abruptly with the breakdown of the nuclear envelope. Chromosomes can now attach to spindle microtubules via their kinetochores and undergo active movement.

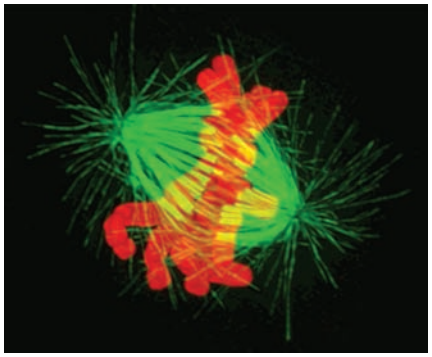


MITOSIS

## 3 METAPHASE

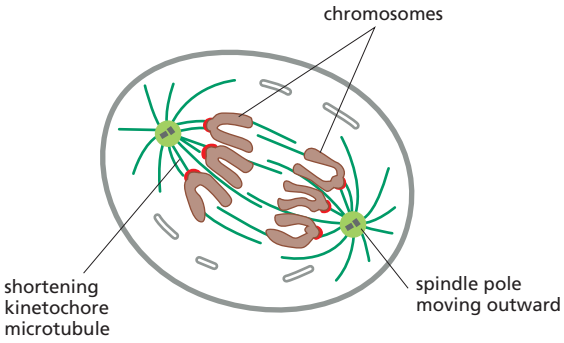



At metaphase, the chromosomes are aligned at the equator of the spindle, midway between the spindle poles. The kinetochore microtubules on each sister chromatid attach to opposite poles of the spindle.

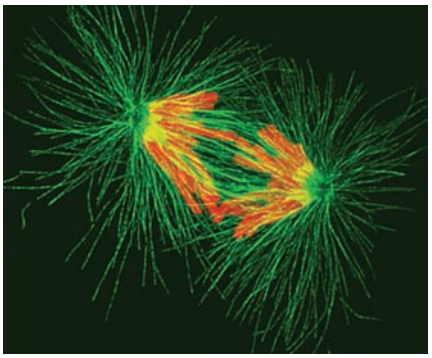


MITOSIS

## 4 ANAPHASE

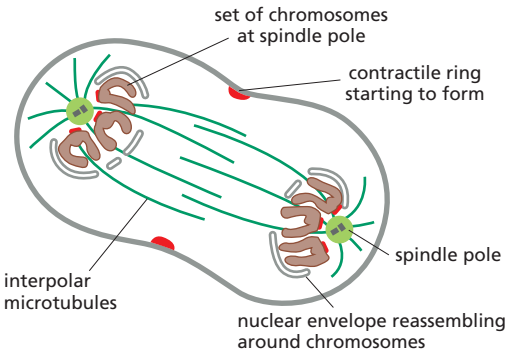



At anaphase, the sister chromatids synchronously separate and are pulled slowly toward the spindle pole to which they are attached. The kinetochore microtubules get shorter, and the spindle poles also move apart, both contributing to chromosome segregation.

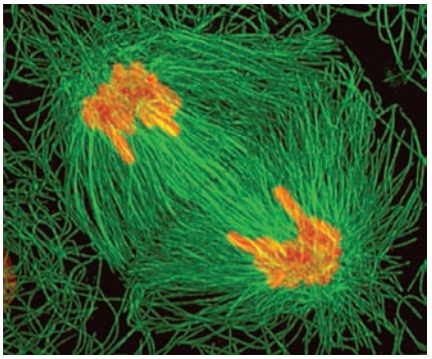


MITOSIS

## 5 TELOPHASE

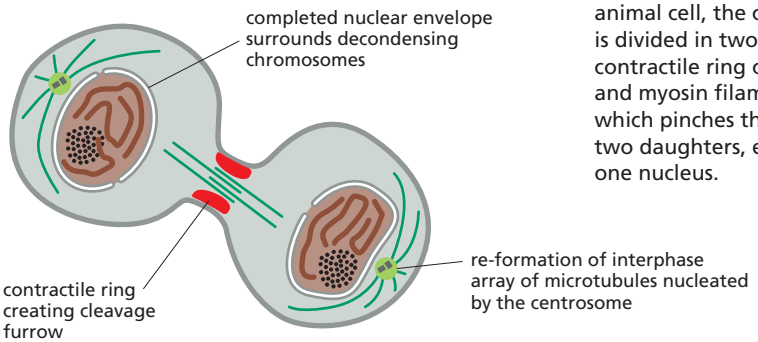



During telophase, the two sets of chromosomes arrive at the poles of the spindle. A new nuclear envelope reassembles around each set, completing the formation of two nuclei and marking the end of mitosis. The division of the cytoplasm begins with the assembly of the contractile ring.

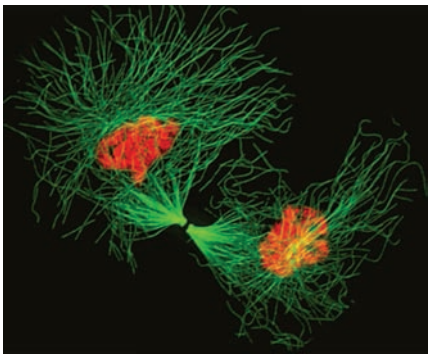


CYTOKINESIS

## CYTOKINESIS




During cytokinesis of an animal cell, the cytoplasm is divided in two by a contractile ring of actin and myosin filaments, which pinches the cell into two daughters, each with one nucleus.



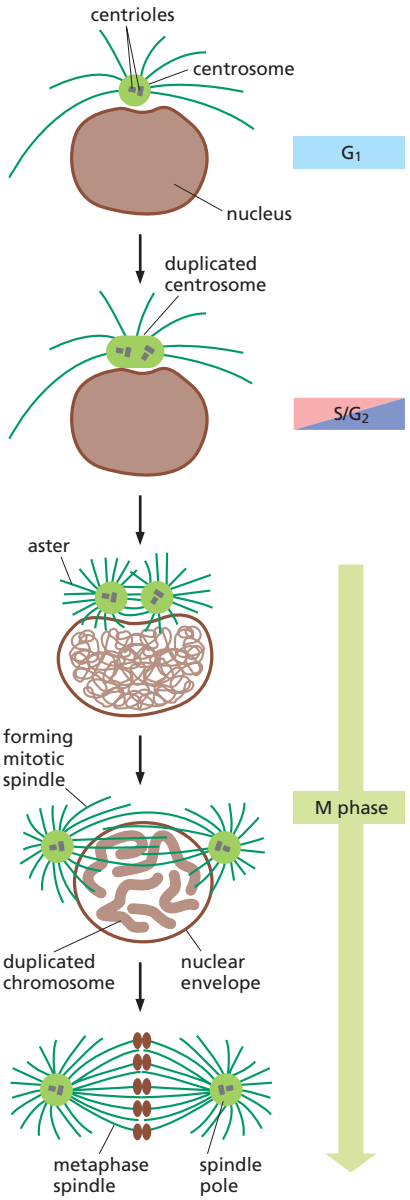



**Figure 18–21 The centrosome in an interphase cell duplicates to form the two poles of a mitotic spindle.** Most animal cells contain a single centrosome, which consists of a pair of centrioles (*gray*) embedded in a matrix of proteins (*light green*). The volume of the centrosome matrix is exaggerated in this diagram for clarity. Although the centrioles are made of a cylindrical array of short microtubules, they do not participate in the nucleation of microtubules from the centrosome (see Figure 17–13). Centrosome duplication begins at the start of S phase and is complete by the end of $G_2$. Initially, the two centrosomes remain together, but, in early M phase, they separate, and each nucleates its own aster of microtubules. The centrosomes then move apart, and the microtubules that interact between the two asters elongate preferentially to form a bipolar mitotic spindle, with an aster at each pole. When the nuclear envelope breaks down, the spindle microtubules are able to interact with the duplicated chromosomes.

Centrosome duplication begins at the same time as DNA replication and the process is triggered by the same Cdks—$G_1$/S-Cdk and S-Cdk—that initiate DNA replication. Initially, when the centrosome duplicates, both copies remain together as a single complex on one side of the nucleus. As mitosis begins, however, the two centrosomes separate, and each nucleates a radial array of microtubules called an **aster**. The two asters move to opposite sides of the nucleus to form the two poles of the mitotic spindle (**Figure 18–21**). The process of centrosome duplication and separation is known as the **centrosome cycle**.

## The Mitotic Spindle Starts to Assemble in Prophase

The mitotic spindle begins to form in **prophase**. The assembly of this highly dynamic structure depends on the remarkable properties of microtubules. As discussed in Chapter 17, microtubules continuously polymerize and depolymerize by the addition and loss of their tubulin subunits, and individual filaments alternate between growing and shrinking—a process called *dynamic instability* (see Figure 17–14). At the start of mitosis, dynamic stability rises—in part because M-Cdk phosphorylates microtubule-associated proteins that influence microtubule stability. As a result, during prophase, rapidly growing and shrinking microtubules extend in all directions from the two centrosomes, exploring the interior of the cell.

Some of the microtubules growing from one centrosome interact with the microtubules from the other centrosome (see Figure 18–21). This interaction stabilizes the microtubules, preventing them from depolymerizing, and it joins the two sets of microtubules together to form the basic framework of the **mitotic spindle**, with its characteristic bipolar shape (**Movie 18.6**). The two centrosomes that give rise to these microtubules are now called **spindle poles**, and the interacting microtubules are called interpolar microtubules (**Figure 18–22**). The assembly of the spindle is driven, in part, by motor proteins associated with the interpolar microtubules that help to cross-link the two sets of microtubules and push the two centrosomes apart.

## Chromosomes Attach to the Mitotic Spindle at Prometaphase

**Prometaphase** starts abruptly with the disassembly of the nuclear envelope, which breaks up into small membrane vesicles. This process is triggered by the phosphorylation and consequent disassembly of nuclear pore proteins and the intermediate filament proteins of the nuclear lamina,

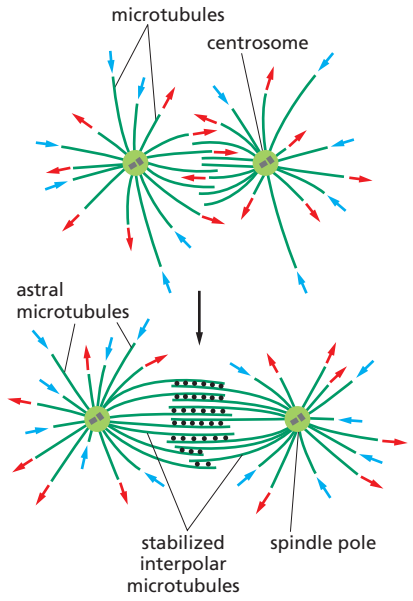



**Figure 18–22 A bipolar mitotic spindle is formed by the selective stabilization of interacting microtubules.** New microtubules grow out in random directions from the two centrosomes. The two ends of a microtubule (by convention, called the plus and the minus ends) have different properties, and it is the minus end that is anchored in the centrosome (discussed in Chapter 17). The free plus ends are dynamically unstable and switch suddenly from uniform growth (outward-pointing *red* arrows) to rapid shrinkage (inward-pointing *blue* arrows). When two microtubules from opposite centrosomes interact in an overlap zone, motor proteins and other microtubule-associated proteins cross-link the microtubules together (*black* dots) in a way that stabilizes the plus ends by decreasing the probability of their depolymerization.

the network of fibrous proteins that underlies and stabilizes the nuclear envelope (see Figure 17–7). The spindle microtubules, which have been lying in wait outside the nucleus, now gain access to the duplicated chromosomes and capture each and everyone (see Panel 18–1, pp. 628–629).

Spindle microtubules attach to the chromosomes at their **kinetochores**, protein complexes that assemble on the centromere of each condensed chromosome during late prophase (**Figure 18–23**). Kinetochores recognize the special DNA sequence that forms a chromosome's centromere: if this sequence is altered, kinetochores fail to assemble and, consequently, the chromosomes fail to segregate properly during mitosis.

Once the nuclear envelope has broken down, a randomly probing microtubule encountering a kinetochore will bind to it, thereby capturing that chromosome and linking it to a spindle pole (see Panel 18–1, pp. 628–629). Of course, each duplicated chromosome has two kinetochores—one on each sister chromatid. Because these sister kinetochores face in opposite directions, they tend to attach to microtubules from opposite poles of

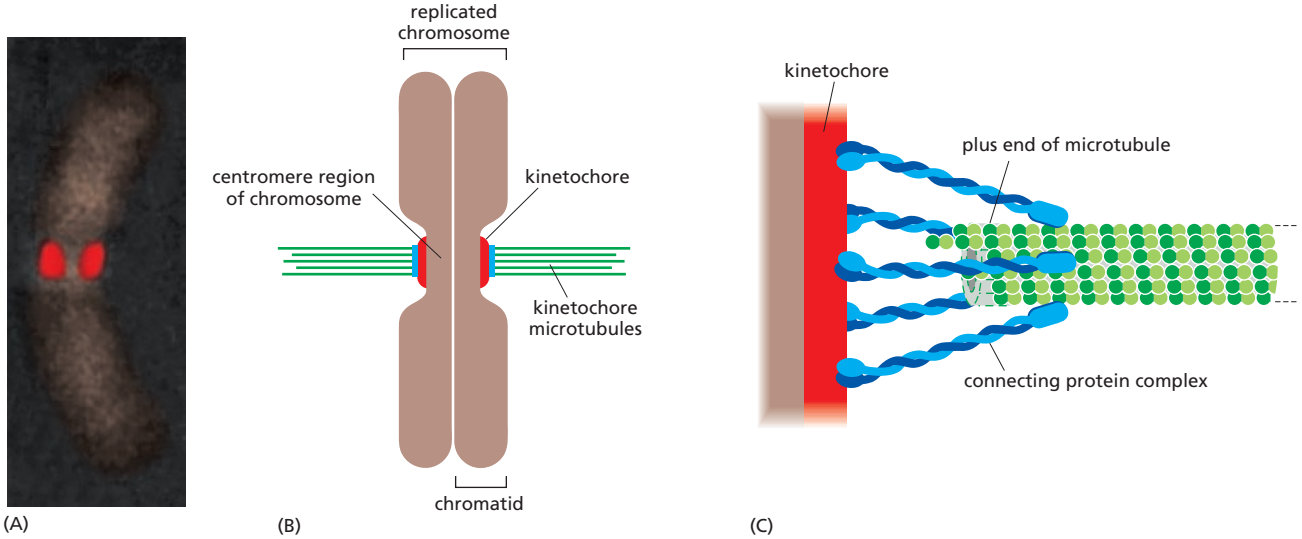



**Figure 18–23 Kinetochores attach chromosomes to the mitotic spindle.** (A) A fluorescence micrograph of a duplicated mitotic chromosome. The kinetochores are stained *red* with fluorescent antibodies that recognize kinetochore proteins. These antibodies come from patients with scleroderma (a disease that causes progressive overproduction of connective tissue in skin and other organs), who, for unknown reasons, produce antibodies against their own kinetochore proteins. (B) Schematic drawing of a mitotic chromosome showing its two sister chromatids attached to kinetochore microtubules, which bind to the kinetochore at their plus ends. Each kinetochore forms a plaque on the surface of the centromere. (C) Each microtubule is attached to the kinetochore via interactions with multiple copies of an elongated connecting protein complex (*blue*). These complexes bind to the sides of the microtubule near its plus end, allowing the microtubule to grow or shrink while remaining attached to the kinetochore. (A, from R.P. Zinkowski et al., *J. Cell Biol.* 113:1091–1110, 1991. With permission from The Rockefeller University Press.)

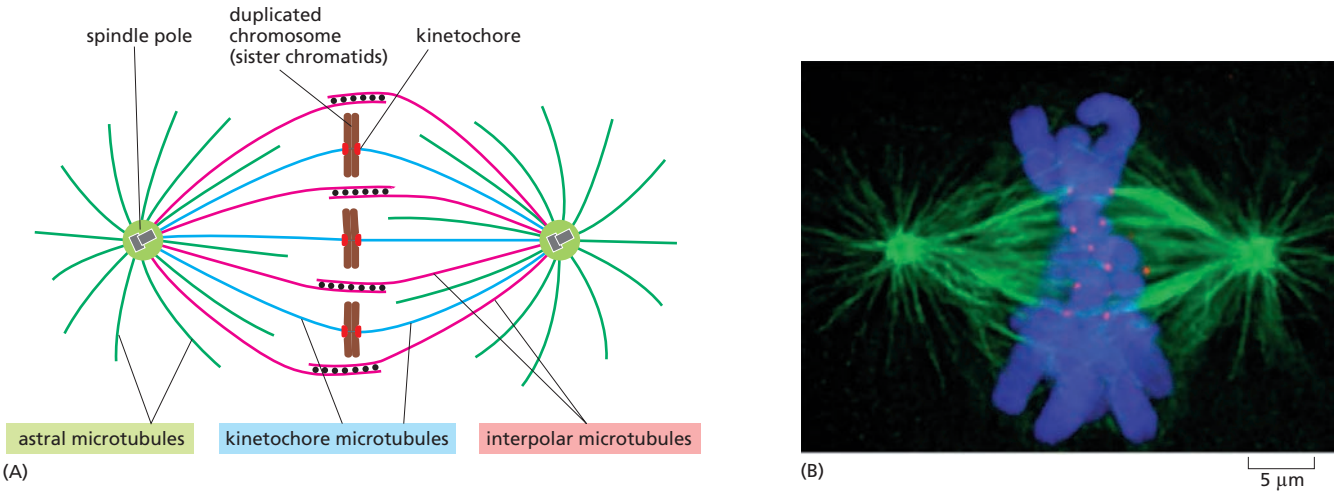



**Figure 18–24 Three classes of microtubules make up the mitotic spindle.** (A) Schematic drawing of a spindle with chromosomes attached, showing the three types of spindle microtubules: astral microtubules, kinetochore microtubules, and interpolar microtubules. In reality, the chromosomes are much larger than shown, and usually multiple microtubules are attached to each kinetochore. (B) Fluorescence micrograph of duplicated chromosomes aligned at the center of the mitotic spindle. In this image, kinetochores are *red* dots, microtubules are *green*, and chromosomes are *blue*. (B, from A. Desai, *Curr. Biol.* 10:R508, 2000. With permission from Elsevier.)

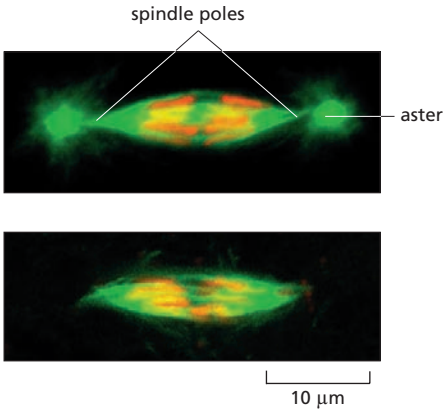



**Figure 18–25 Motor proteins and chromosomes can direct the assembly of a functional bipolar spindle in the absence of centrosomes.** In these fluorescence micrographs of embryos of the insect *Sciara*, the microtubules are stained *green* and the chromosomes *red*. The top micrograph shows a normal spindle formed by centrosomes in a fertilized embryo. The bottom micrograph shows a spindle formed without centrosomes in an embryo that initiated development without fertilization and thus lacks the centrosome normally provided by the sperm when it fertilizes the egg. Note that the spindle with centrosomes has an aster at each pole, whereas the spindle formed without centrosomes does not. As shown, both types of spindles are able to segregate chromosomes. (From B. de Saint Phalle and W. Sullivan, *J. Cell Biol.* 141:1383–1391, 1998. With permission from The Rockefeller University Press.)

the spindle; thus, each duplicated chromosome becomes linked to both spindle poles. The attachment to opposite poles, called **bi-orientation**, generates tension on the kinetochores, which are being pulled in opposite directions. This tension signals to the sister kinetochores that they are attached correctly and are ready to be separated (Movie 18.7). The cell-cycle control system monitors this tension to ensure correct chromosome attachment (see Figure 18–3), a safeguard we discuss in detail shortly.

The number of microtubules attached to each kinetochore varies among species: each human kinetochore binds 20–40 microtubules, for example, whereas a yeast kinetochore binds just one. The three classes of microtubules that form the mitotic spindle are highlighted in Figure 18–24.

## Chromosomes Assist in the Assembly of the Mitotic Spindle

Chromosomes are more than passive passengers in the process of spindle assembly: they themselves can stabilize and organize microtubules into functional mitotic spindles. In cells without centrosomes—including some animal cell types and all plant cells—the chromosomes nucleate microtubule assembly, and motor proteins then move and arrange the microtubules and chromosomes into a bipolar spindle. Even in animal cells that normally have centrosomes, a bipolar spindle can still be formed in this way if the centrosomes are removed (Figure 18–25). In cells with centrosomes, the chromosomes, motor proteins, and centrosomes work together to form the mitotic spindle.

## Chromosomes Line Up at the Spindle Equator at Metaphase

During prometaphase, the duplicated chromosomes, now attached to the mitotic spindle, begin to move about, as if jerked first this way and then that. Eventually, they align at the equator of the spindle, halfway between the two spindle poles, thereby forming the *metaphase plate*. This event defines the beginning of **metaphase** (see Figure 18–24B and

Figure 18–26). Although the forces that act to bring the chromosomes to the equator are not completely understood, both the continual growth and shrinkage of the microtubules and the action of microtubule motor proteins are required. A continuous balanced addition and loss of tubulin subunits is also required to maintain the metaphase spindle: when tubulin addition to the ends of microtubules is blocked by the drug colchicine, tubulin loss continues until the metaphase spindle disappears.

The chromosomes gathered at the equator of the metaphase spindle oscillate back and forth, continually adjusting their positions, indicating that the tug-of-war between the microtubules attached to opposite poles of the spindle continues to operate after the chromosomes are all aligned. If the kinetochore attachments on one side of a duplicated chromosome are artificially severed with a laser beam during metaphase, the entire chromosome immediately moves toward the pole to which it remains attached. Similarly, if the attachment between sister chromatids is cut, the two chromosomes separate and move toward opposite poles. These experiments show that the duplicated chromosomes are not simply deposited at the metaphase plate. They are suspended there under tension. In anaphase, that tension will pull the sister chromatids apart.

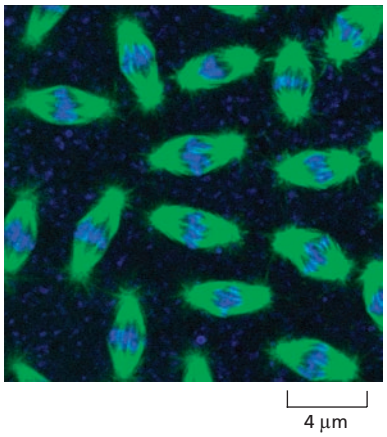



**Figure 18–26 During metaphase, duplicated chromosomes gather halfway between the two spindle poles.** This fluorescence micrograph shows multiple mitotic spindles at metaphase in a fruit fly (*Drosophila*) embryo. The microtubules are stained *green*, and the chromosomes are stained *blue*. At this stage of *Drosophila* development, there are multiple nuclei in one large cytoplasmic compartment, and all of the nuclei divide synchronously, which is why all of the nuclei shown here are at the same metaphase stage of the cell cycle (Movie 18.8). Metaphase spindles are usually pictured in two dimensions, as they are here; when viewed in three dimensions, however, the chromosomes are seen to be gathered at a platelike region at the equator of the spindle—the so-called metaphase plate. (Courtesy of William Sullivan.)

## Proteolysis Triggers Sister-Chromatid Separation at Anaphase

**Anaphase** begins abruptly with the breakage of the cohesin linkages that hold together the sister chromatids in a duplicated chromosome (see Figure 18–18A). This release allows each chromosome to be pulled toward the spindle pole to which it is attached (Figure 18–27). The movement segregates the two identical sets of chromosomes to opposite ends of the spindle (see Panel 18–1, pp. 628–629).

The cohesin linkage is destroyed by a protease called *separase*. Before anaphase begins, this protease is held in an inactive state by an inhibitory protein called *securin*. At the beginning of anaphase, securin is targeted for destruction by APC/C—the same protein complex, discussed earlier, that marks M cyclin for degradation. Once securin has been removed, separase is then free to sever the cohesin linkages (Figure 18–28).

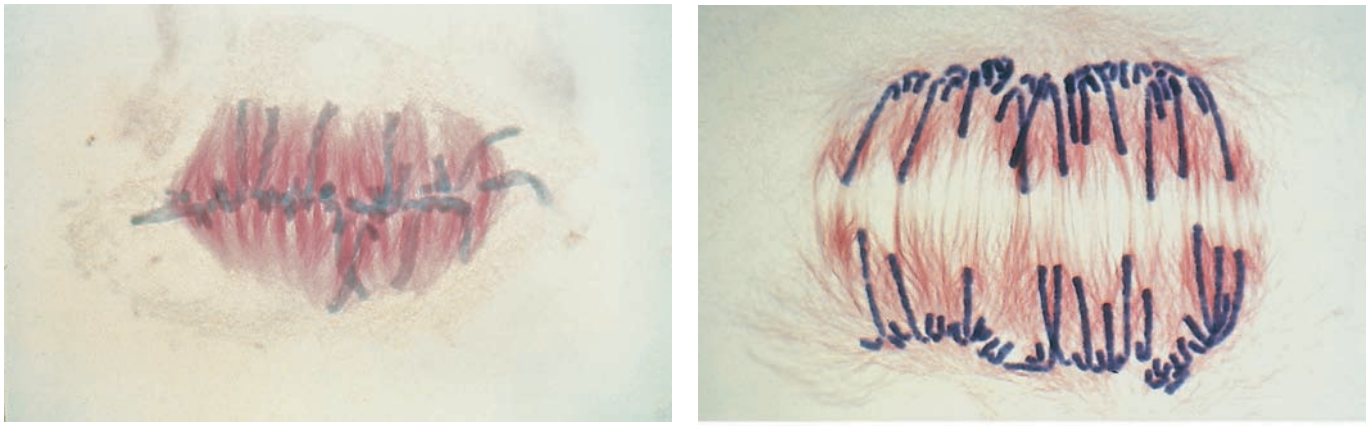



**Figure 18–27 Sister chromatids separate at anaphase.** In the transition from (A) metaphase to (B) anaphase, the sister chromatids of duplicated chromosomes (stained *blue*) suddenly separate, allowing the chromosomes to move toward opposite poles, as seen in these plant cells stained with gold-labeled antibodies to label the microtubules (*red*). Plant cells generally do not have centrosomes and therefore have less sharply defined spindle poles than do animal cells (see also Figure 18–35); nonetheless, spindle poles are present here at the top and bottom of each micrograph, although they cannot be seen. (Courtesy of Andrew Bajer.)

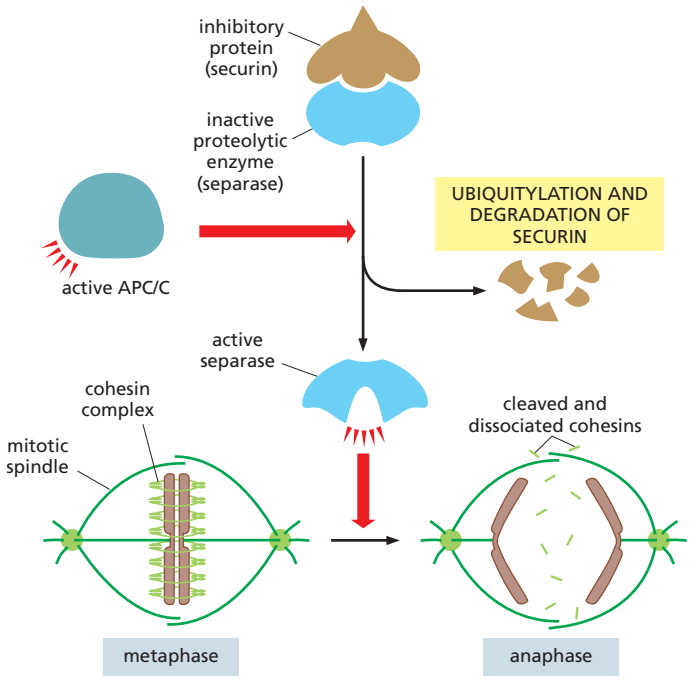



**Figure 18–28 APC/C triggers the separation of sister chromatids by promoting the destruction of cohesins.** APC/C indirectly triggers the cleavage of the cohesins that hold sister chromatids together. It catalyzes the ubiquitylation and destruction of an inhibitory protein called securin, which blocks the activation of a proteolytic enzyme called separase. When freed from securin, separase cleaves the cohesin complexes, allowing the mitotic spindle to pull the sister chromatids apart.

## Chromosomes Segregate During Anaphase

Once the sister chromatids separate, they all move toward the spindle poles at the same speed, which is typically about 1 μm per minute. The movement is the consequence of two independent and overlapping processes that rely on different parts of the mitotic spindle. In anaphase A, the kinetochore microtubules shorten and the attached chromosomes move poleward. In anaphase B, the spindle poles themselves move apart, further segregating the two sets of chromosomes (**Figure 18–29**).

The driving force for the movements of anaphase A is thought to be provided mainly by the loss of tubulin subunits from both ends of the kinetochore microtubules. The driving forces in anaphase B are thought to be provided by two sets of motor proteins—members of the kinesin and dynein families—operating on different types of spindle microtubules (see Figure 17–19A). Kinesin proteins act on the long, overlapping interpolar microtubules, sliding the microtubules from opposite poles past one another at the equator of the spindle and pushing the spindle poles apart. Dynein proteins, anchored to the plasma membrane, move along astral microtubules to pull the poles apart (see Figure 18–29B).

## An Unattached Chromosome Will Prevent Sister-Chromatid Separation

If a dividing cell were to begin to segregate its chromosomes before all the chromosomes were properly attached to the spindle, one daughter cell would receive an incomplete set of chromosomes, while the other would receive a surplus. Both situations could be lethal. Thus, a dividing cell must ensure that every last chromosome is attached properly to the spindle before it completes mitosis. To monitor chromosome attachment, the cell makes use of a negative signal: the kinetochores of unattached chromosomes send a "stop" signal to the cell-cycle control system. This signal inhibits further progress through mitosis by blocking the activation of APC/C (see Figure 18–28). Without active APC/C, the sister chromatids

## QUESTION 18–6

If fine glass needles are used to manipulate a chromosome inside a living cell during early M phase, it is possible to trick the kinetochores on the two sister chromatids into attaching to the same spindle pole. This arrangement is normally unstable, but the attachments can be stabilized if the needle is used to gently pull the chromosome so that the microtubules attached to both kinetochores (via the same spindle pole) are under tension. What does this suggest to you about the mechanism by which kinetochores normally become attached and stay attached to microtubules from opposite spindle poles? Is the finding consistent with the possibility that a kinetochore is programmed to attach to microtubules from a particular spindle pole? Explain your answers.

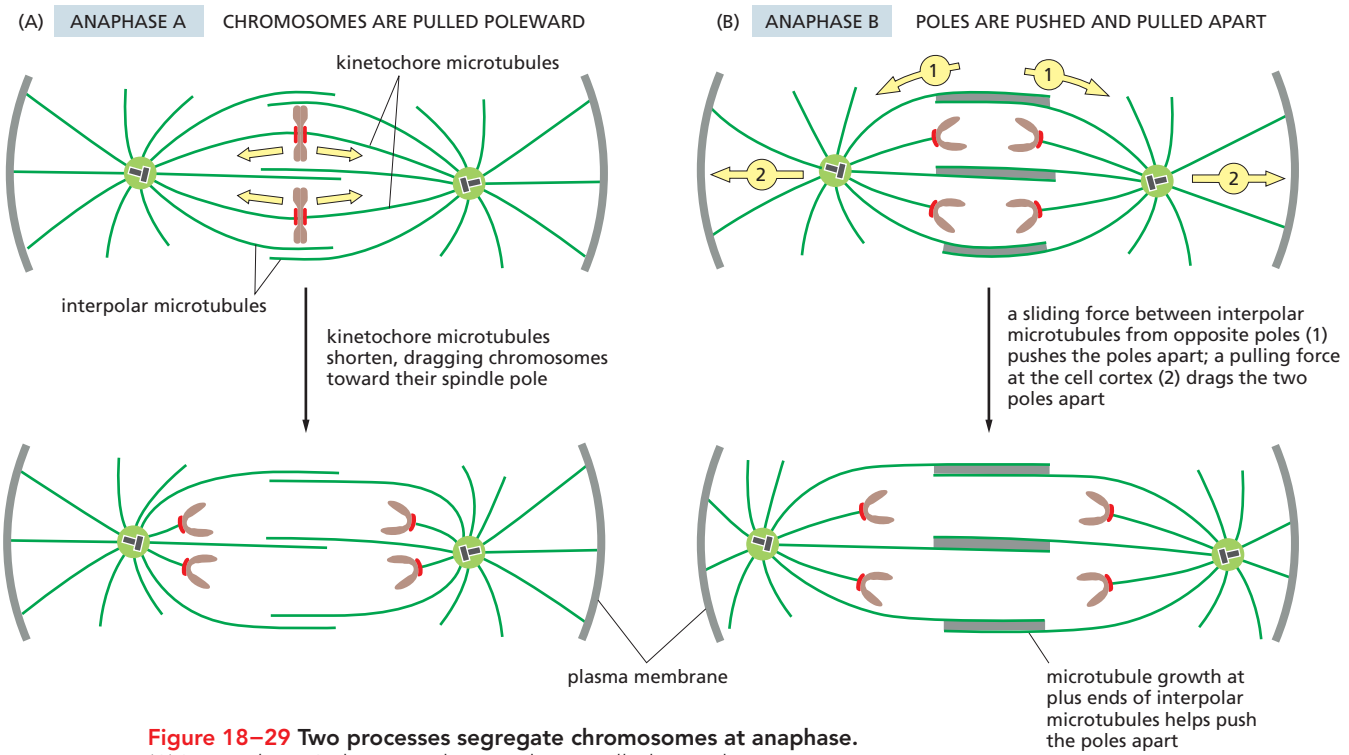



**Figure 18–29 Two processes segregate chromosomes at anaphase.** (A) In *anaphase A*, the sister chromatids are *pulled* toward opposite poles as the kinetochore microtubules depolymerize. The force driving this movement is generated mainly at the kinetochore. (B) In *anaphase B*, the two spindle poles move apart as the result of two separate forces: (1) the elongation and sliding of the interpolar microtubules past one another *pushes* the two poles apart, and (2) forces exerted on the outward-pointing astral microtubules at each spindle pole *pull* the poles away from each other, toward the cell cortex. Both forces are thought to depend on the action of motor proteins associated with the microtubules.

remain glued together. Thus, none of the duplicated chromosomes can be pulled apart until every chromosome has been positioned correctly on the mitotic spindle. The absence of APC/C also prevents the destruction of cyclins (see Figure 18–9), so that Cdks remain active—thus prolonging mitosis. This *spindle assembly checkpoint* thereby controls the onset of anaphase, as well as the exit from mitosis, as mentioned earlier (see Figure 18–12).

## The Nuclear Envelope Re-forms at Telophase

By the end of anaphase, the chromosomes have separated into two equal groups, one at each pole of the spindle. During **telophase**, the final stage of mitosis, the mitotic spindle disassembles, and a nuclear envelope reassembles around each group of chromosomes to form the two daughter nuclei (Movie 18.9 and Movie 18.10). Vesicles of nuclear membrane associate with the clustered chromosomes and then fuse to re-form the nuclear envelope (see Panel 18–1, pp. 628–629). During this process, the nuclear pore proteins and nuclear lamins that were phosphorylated during prometaphase are now dephosphorylated, which allows them to reassemble and rebuild the nuclear envelope and lamina (Figure 18–30). Once the nuclear envelope has been re-established, the pores restore the localization of cytosolic and nuclear proteins and the condensed chromosomes decondense into their interphase state. A new nucleus has been created, and mitosis is complete. All that remains is for the cell to complete its division into two separate daughter cells.

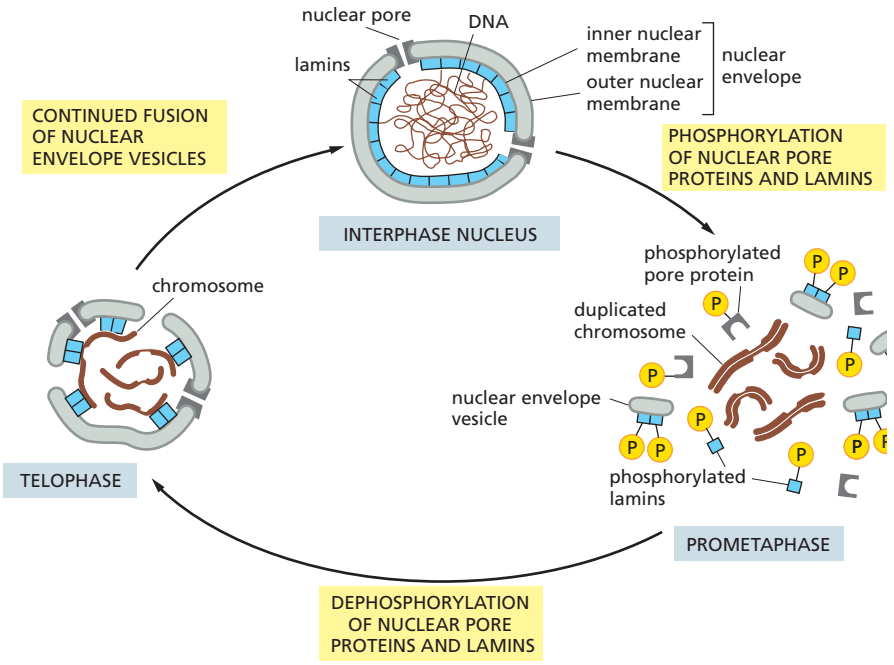



**Figure 18–30 The nuclear envelope breaks down and re-forms during mitosis.** The phosphorylation of nuclear pore proteins and lamins helps trigger the disassembly of the nuclear envelope at prometaphase. Dephosphorylation of these proteins at telophase helps reverse the process.

## QUESTION 18–7

Consider the events that lead to the formation of the new nucleus at telophase. How do nuclear and cytosolic proteins become properly re-sorted so that the new nucleus contains nuclear proteins but not cytosolic proteins?

## CYTOKINESIS

**Cytokinesis**, the process by which the cytoplasm is cleaved in two, completes M phase. It usually begins in anaphase but is not completed until after the two daughter nuclei have re-formed in telophase. Whereas mitosis depends on a transient microtubule-based structure, the mitotic spindle, cytokinesis in animal cells depends on a transient structure based on actin and myosin filaments, the *contractile ring* (see Figure 18–19). Both the plane of cleavage and the timing of cytokinesis, however, are determined by the mitotic spindle.

### The Mitotic Spindle Determines the Plane of Cytoplasmic Cleavage

The first visible sign of cytokinesis in animal cells is a puckering and furrowing of the plasma membrane that occurs during anaphase (**Figure 18–31**). The furrowing invariably occurs along a plane that runs perpendicular to the long axis of the mitotic spindle. This positioning ensures that the *cleavage furrow* cuts between the two groups of segregated chromosomes, so that each daughter cell receives an identical and complete set of chromosomes. If the mitotic spindle is deliberately displaced (using

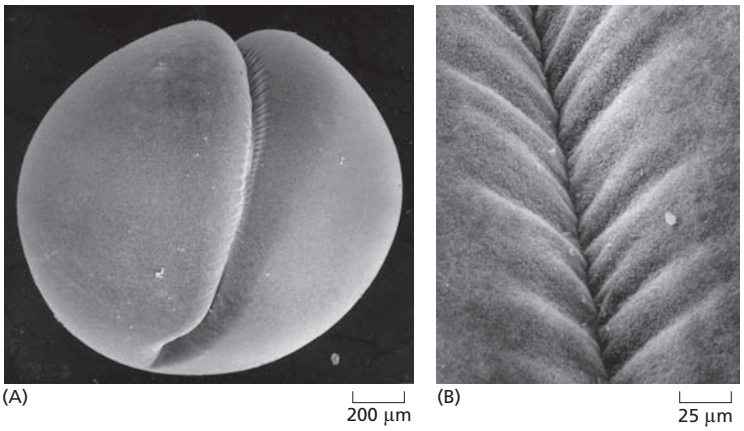



**Figure 18–31 The cleavage furrow is formed by the action of the contractile ring underneath the plasma membrane.** In these scanning electron micrographs of a dividing fertilized frog egg, the cleavage furrow is unusually well defined. (A) Low-magnification view of the egg surface. (B) A higher-magnification view of the cleavage furrow. (From H.W. Beams and R.G. Kessel, *Am. Sci.* 64:279–290, 1976. With permission of Sigma Xi.)

a fine glass needle) as soon as the furrow appears, the furrow will disappear and a new one will develop at a site corresponding to the new spindle location and orientation. Once the furrowing process is well under way, however, cleavage proceeds even if the mitotic spindle is artificially sucked out of the cell or depolymerized using the drug colchicine.

How does the mitotic spindle dictate the position of the cleavage furrow? The mechanism is still uncertain, but it appears that, during anaphase, the overlapping interpolar microtubules that form the *central spindle* recruit and activate proteins that signal to the cell cortex to initiate the assembly of the contractile ring at a position midway between the spindle poles (**Figure 18–32**). Because these signals originate during anaphase, this mechanism also contributes to the timing of cytokinesis in late mitosis.

When the mitotic spindle is located centrally in the cell—the usual situation in most dividing cells—the two daughter cells will be of equal size. During embryonic development, however, there are some instances in which the dividing cell moves its mitotic spindle to an asymmetrical position, and, consequently, the furrow creates two daughter cells that differ in size. In most of these *asymmetric divisions*, the daughters also differ in the molecules they inherit, and they usually develop into different cell types.

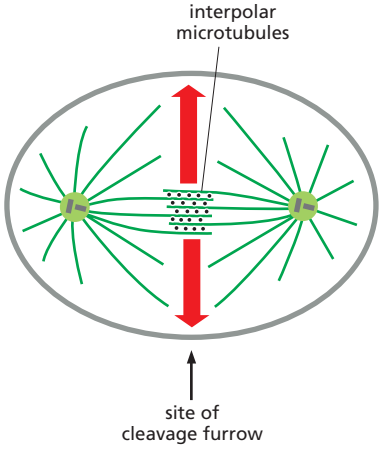
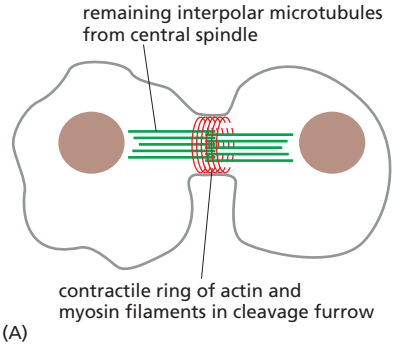



**Figure 18–32 Position of the cleavage furrow is dictated by the central spindle.** In this model, the interpolar microtubules recruit proteins that generate a signal (*red* arrows) that activates a protein called RhoA in the cell cortex. RhoA, a member of the Rho family of GTPases discussed in Chapter 17, controls the assembly and contraction of the contractile ring midway between the spindle poles.

## The Contractile Ring of Animal Cells Is Made of Actin and Myosin Filaments

The **contractile ring** is composed mainly of an overlapping array of actin filaments and myosin filaments (**Figure 18–33**). It assembles at anaphase and is attached to membrane-associated proteins on the cytosolic face of the plasma membrane. Once assembled, the contractile ring is capable of exerting a force strong enough to bend a fine glass needle inserted into the cell before cytokinesis. Much of this force is generated by the sliding of the actin filaments against the myosin filaments. Unlike the stable association of actin and myosin filaments in muscle fibers, however, the contractile ring is a dynamic and transient structure: it assembles to carry out cytokinesis, gradually becomes smaller as cytokinesis progresses, and disassembles completely once the cell has been cleaved in two.

Cell division in many animal cells is accompanied by large changes in cell shape and a decrease in the adherence of the cell to its neighbors, to the extracellular matrix, or to both. These changes result, in part, from the reorganization of actin and myosin filaments in the cell cortex, only one aspect of which is the assembly of the contractile ring. Mammalian fibroblasts in culture, for example, spread out flat during interphase, as a result of the strong adhesive contacts they make with the surface they are growing on—called the *substratum*. As the cells enter M phase, however, they round up. This change in shape takes place, in part, because some of the plasma membrane proteins responsible for attaching the cells to the substratum—the *integrins* (discussed in Chapter 20)—become phosphor-ylated and thus weaken their grip. Once cytokinesis is complete, the daughter cells reestablish their strong contacts with the substratum and



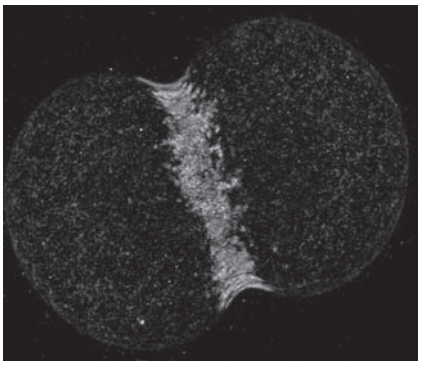




**Figure 18–33 The contractile ring divides the cell in two.** (A) Schematic diagram of the midregion of a dividing cell showing the contractile ring beneath the plasma membrane and the remains of the two sets of interpolar microtubules. (B) In this dividing sea urchin embryo, the contractile ring is revealed by staining with a fluorescently labeled antibody that binds to myosin. (B, courtesy of George von Dassow.)

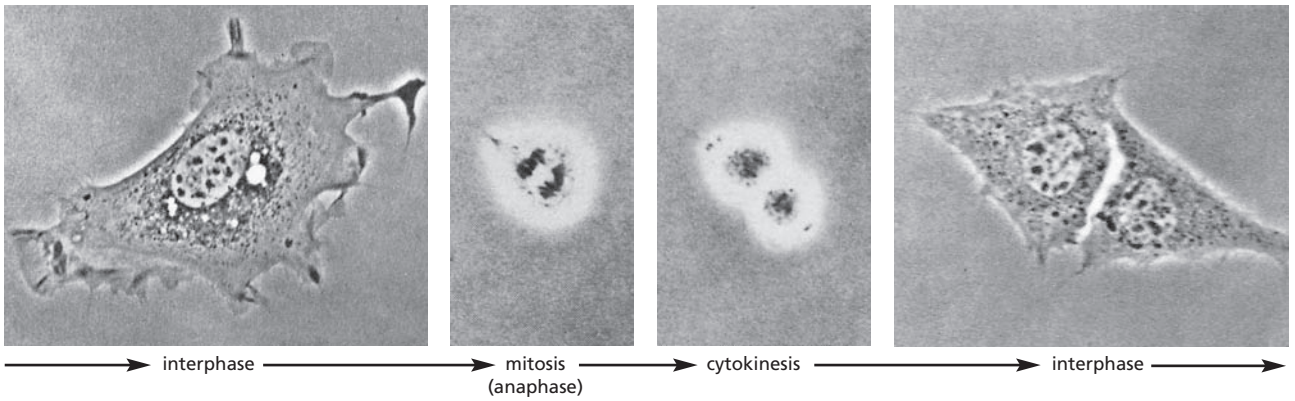



**Figure 18–34 Animal cells change shape during M phase.** In these micrographs of a mouse fibroblast dividing in culture, the same cell was photographed at successive times. Note how the cell becomes smaller and rounded as it enters mitosis; the two daughter cells then flatten out again after cytokinesis is complete. (Courtesy of Guenter Albrecht-Buehler.)

flatten out again (**Figure 18–34**). When cells divide in an animal tissue, this cycle of attachment and detachment presumably allows the cells to rearrange their contacts with neighboring cells and with the extracellular matrix, so that the new cells produced by cell division can be accommodated within the tissue.

## Cytokinesis in Plant Cells Involves the Formation of a New Cell Wall

The mechanism of cytokinesis in higher plants is entirely different from that in animal cells, presumably because plant cells are surrounded by a tough cell wall (discussed in Chapter 20). The two daughter cells are separated not by the action of a contractile ring at the cell surface but instead by the construction of a new wall that forms inside the dividing cell. The positioning of this new wall precisely determines the position of the two daughter cells relative to neighboring cells. Thus, the planes of cell division, together with cell enlargement, determine the final form of the plant.

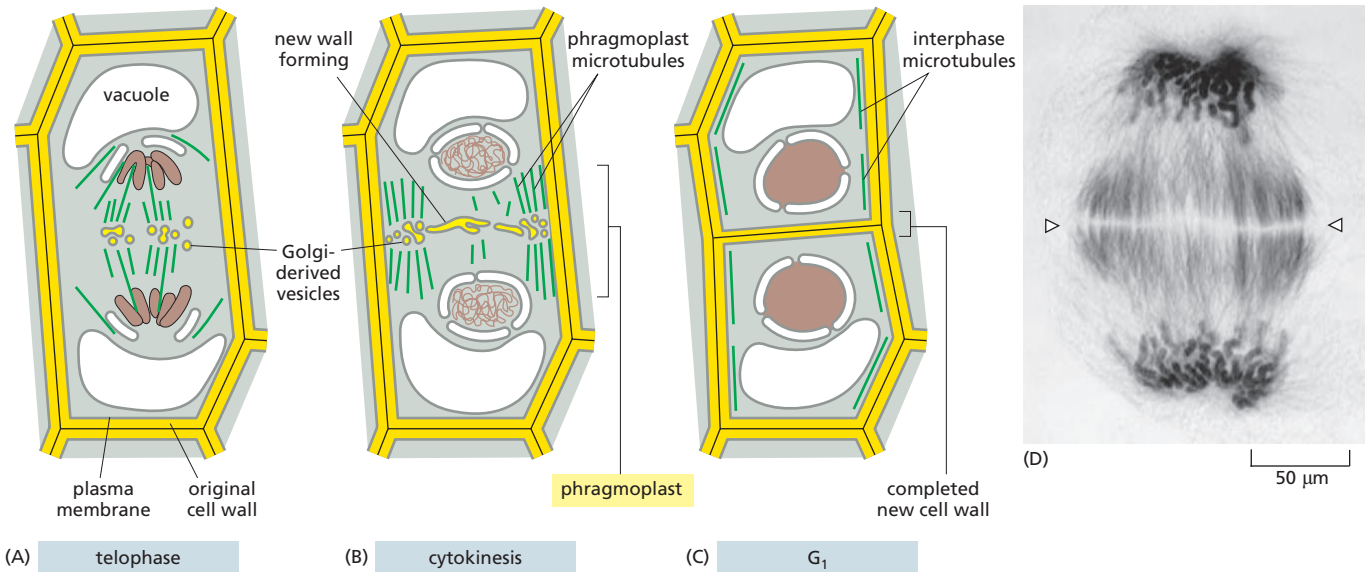
The new cell wall starts to assemble in the cytoplasm between the two sets of segregated chromosomes at the start of telophase. The assembly process is guided by a structure called the **phragmoplast**, which is formed by the remains of the interpolar microtubules at the equator of the old mitotic spindle. Small membrane-enclosed vesicles, largely derived from the Golgi apparatus and filled with polysaccharides and glycoproteins required for the cell wall matrix, are transported along the microtubules to the phragmoplast. Here, they fuse to form a disclike, membrane-enclosed structure, which expands outward by further vesicle fusion until it reaches the plasma membrane and original cell wall, thereby dividing the cell in two (**Figure 18–35**). Later, cellulose microfibrils are laid down within the matrix to complete the construction of the new cell wall.

## Membrane-enclosed Organelles Must Be Distributed to Daughter Cells When a Cell Divides

Organelles such as mitochondria and chloroplasts cannot assemble spontaneously from their individual components; they arise only from the growth and division of the preexisting organelles. Likewise, endoplasmic reticulum (ER) and Golgi apparatus also derive from preexisting organelle fragments. How, then, are these various membrane-enclosed organelles segregated when the cell divides so that each daughter gets its share?

### QUESTION 18–8

Draw a detailed view of the formation of the new cell wall that separates the two daughter cells when a plant cell divides (see Figure 18–35). In particular, show where the membrane proteins of the Golgi-derived vesicles end up, indicating what happens to the part of a protein in the Golgi vesicle membrane that is exposed to the interior of the Golgi vesicle. (Refer to Chapter 11 if you need a reminder of membrane structure.)




Mitochondria and chloroplasts are usually present in large numbers and will be safely inherited if, on average, their numbers simply double once each cell cycle. The ER in interphase cells is continuous with the nuclear membrane and is organized by the microtubule cytoskeleton (see Figure 17–21). Upon entry into M phase, the reorganization of the microtubules releases the ER; in most cells, the released ER remains intact during mitosis and is cut in two during cytokinesis. The Golgi apparatus fragments during mitosis; the fragments associate with the spindle microtubules via motor proteins, thereby hitching a ride into the daughter cells as the spindle elongates in anaphase. Other components of the cell—including the other membrane-enclosed organelles, ribosomes, and all of the soluble proteins—are inherited randomly when the cell divides.

Having discussed how cells divide, we now turn to the general problem of how the size of an animal or an organ is determined, which leads us to consider how cell number and cell size are controlled.

## CONTROL OF CELL NUMBERS AND CELL SIZE

A fertilized mouse egg and a fertilized human egg are similar in size—about 100 μm in diameter. Yet an adult mouse is much smaller than an adult human. What are the differences between the control of cell behavior in humans and mice that generate such big differences in size? The same fundamental question can be asked about each organ and tissue in an individual's body. What adjustment of cell behavior explains the length of an elephant's trunk or the size of its brain or its liver? These questions are largely unanswered, but it is at least possible to say what the ingredients of an answer must be. Three fundamental processes largely determine organ and body size: cell growth, cell division, and cell death. Each of these processes, in turn, depends on programs intrinsic to the individual cell, regulated by signals from other cells in the body.

In this section, we first consider how organisms eliminate unwanted cells by a form of programmed cell death called *apoptosis*. We then discuss how extracellular signals balance cell death, cell growth, and cell division—thereby helping control the size of an animal and its organs. We conclude the section with a brief discussion of the extracellular signals that control these three processes.

**Figure 18–35 Cytokinesis in a plant cell is guided by a specialized microtubule-based structure called the phragmoplast.** (A) At the beginning of telophase, after the chromosomes have segregated, a new cell wall starts to assemble inside the cell at the equator of the old spindle. (B) The interpolar microtubules of the mitotic spindle remaining at telophase form the *phragmoplast* and guide vesicles, derived from the Golgi apparatus, toward the equator of the spindle. The vesicles, which are filled with cell wall material, fuse to form the growing new cell wall that grows outward to reach the plasma membrane and original cell wall. (C) The preexisting plasma membrane and the membrane surrounding the new cell wall then fuse, completely separating the two daughter cells. (D) A light micrograph of a plant cell in telophase is shown at a stage corresponding to (A). The cell has been stained to show both the microtubules and the two sets of chromosomes segregated at the two poles of the spindle. The location of the growing new cell wall is indicated by the arrowheads. (D, courtesy of Andrew Bajer.)

### QUESTION 18–9

The Golgi apparatus is thought to be partitioned into the daughter cells at cell division by a random distribution of fragments that are created at mitosis. Explain why random partitioning of chromosomes would not work.

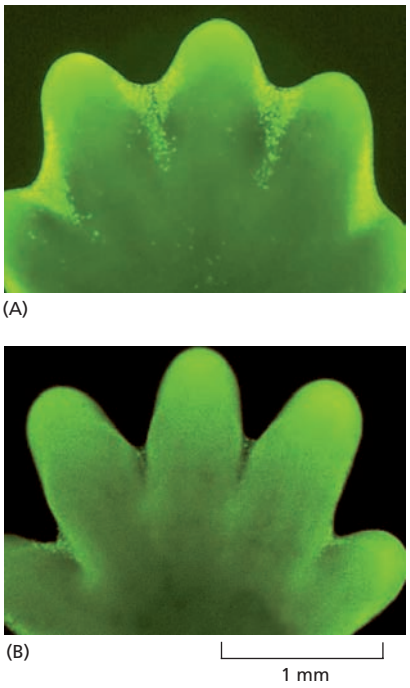



**Figure 18–36 Apoptosis in the developing mouse paw sculpts the digits.** (A) The paw in this mouse embryo has been stained with a dye that specifically labels cells that have undergone apoptosis. The apoptotic cells appear as *bright green dots* between the developing digits. (B) This cell death eliminates the tissue between the developing digits, as seen in the paw shown one day later. Here, few, if any, apoptotic cells can be seen—demonstrating how quickly apoptotic cells can be cleared from a tissue. (From W. Wood et al., *Development* 127:5245–5252, 2000. With permission from The Company of Biologists Ltd.)

## Apoptosis Helps Regulate Animal Cell Numbers

The cells of a multicellular organism are members of a highly organized community. The number of cells in this community is tightly regulated—not simply by controlling the rate of cell division, but also by controlling the rate of cell death. If cells are no longer needed, they can remove themselves by activating an intracellular suicide program—a process called **programmed cell death**. In animals, the most common form of programmed cell death is called **apoptosis** (from a Greek word meaning "falling off," as leaves fall from a tree).

The amount of apoptosis that occurs in both developing and adult animal tissues can be astonishing. In the developing vertebrate nervous system, for example, more than half of some types of nerve cells normally die soon after they are formed. In a healthy adult human, billions of cells in the bone marrow and intestine perish every hour. It seems remarkably wasteful for so many cells to die, especially as the vast majority are perfectly healthy at the time they kill themselves. What purposes does this massive cell suicide serve?

In some cases, the answers are clear. Mouse paws—and our own hands and feet—are sculpted by apoptosis during embryonic development: they start out as spadelike structures, and the individual fingers and toes separate because the cells between them die (**Figure 18–36**). In other cases, cells die when the structure they form is no longer needed. When a tadpole changes into a frog at metamorphosis, the cells in its tail die, and the tail, which is not needed in the adult frog, disappears (**Figure 18–37**). In these cases, the unneeded cells die largely through apoptosis.

In adult tissues, cell death usually balances cell division, unless the tissue is growing or shrinking. If part of the liver is removed in an adult rat, for example, liver cells proliferate to make up the loss. Conversely, if a rat is treated with the drug phenobarbital, which stimulates liver cell division, the liver enlarges. However, when the phenobarbital treatment is stopped, apoptosis in the liver greatly increases until the organ has returned to its original size, usually within a week or so. Thus, the liver is kept at a constant size through regulation of both the rate of cell death and the rate of cell birth.

## Apoptosis Is Mediated by an Intracellular Proteolytic Cascade

Cells that die as a result of acute injury typically swell and burst, spewing their contents across their neighbors, a process called *cell necrosis* (**Figure 18–38A**). This eruption triggers a potentially damaging inflammatory response. By contrast, a cell that undergoes apoptosis dies neatly, without damaging its neighbors. A cell in the throes of apoptosis may develop irregular bulges—or *blebs*—on its surface; but it then shrinks and condenses (**Figure 18–38B**). The cytoskeleton collapses, the nuclear envelope disassembles, and the nuclear DNA breaks up into fragments (**Movie 18.11**). Most importantly, the cell surface is altered in such a manner that it immediately attracts phagocytic cells, usually specialized

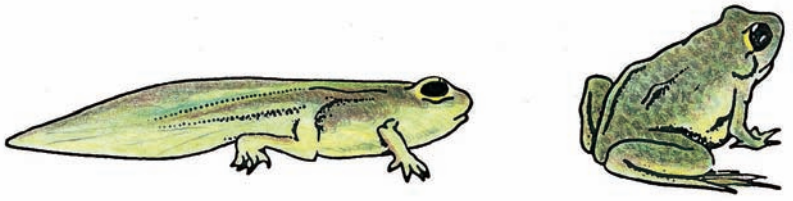


**Figure 18–37 As a tadpole changes into a frog, the cells in its tail are induced to undergo apoptosis.** All of the changes that occur during metamorphosis, including the induction of apoptosis in the tadpole tail, are stimulated by an increase in thyroid hormone in the blood.

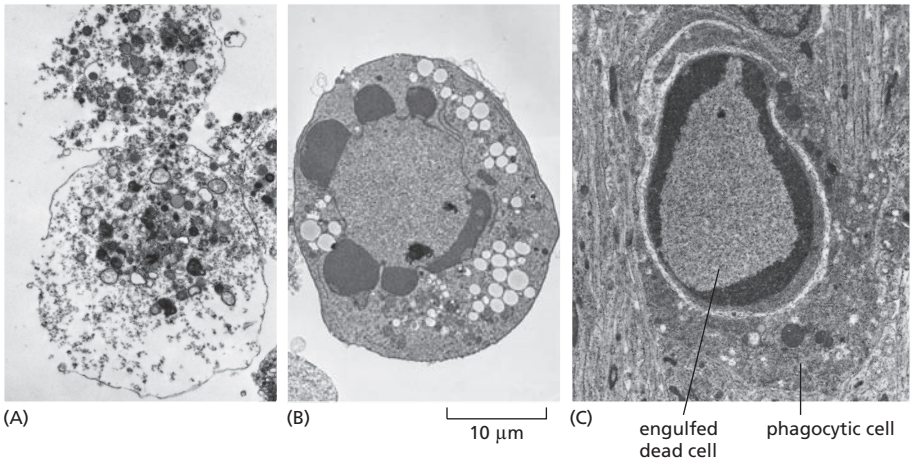



**Figure 18–38 Cells undergoing apoptosis die quickly and cleanly.** Electron micrographs showing cells that have died (A) by necrosis or (B and C) by apoptosis. The cells in (A) and (B) died in a culture dish, whereas the cell in (C) died in a developing tissue and has been engulfed by a phagocytic cell. Note that the cell in (A) seems to have exploded, whereas those in (B) and (C) have condensed but seem relatively intact. The large vacuoles seen in the cytoplasm of the cell in (B) are a variable feature of apoptosis. (Courtesy of Julia Burne.)

phagocytic cells called macrophages (see Figure 15–32B). These cells engulf the apoptotic cell before its contents can leak out (**Figure 18–38C**). This rapid removal of the dying cell avoids the damaging consequences of cell necrosis, and it also allows the organic components of the apoptotic cell to be recycled by the cell that ingests it.

The molecular machinery responsible for apoptosis, which seems to be similar in most animal cells, involves a family of proteases called **caspases**. These enzymes are made as inactive precursors, called *procaspases*, which are activated in response to signals that induce apoptosis. Two types of caspases work together to take a cell apart. *Initiator caspases* cleave, and thereby activate, downstream *executioner caspases*, which dismember numerous key proteins in the cell (**Figure 18–39**). One executioner caspase, for example, targets the lamin proteins that form the nuclear lamina underlying the nuclear envelope (see Figure 18–30); this cleavage causes the irreversible breakdown of the nuclear lamina, which allows nucleases to enter the nucleus and break down the DNA.

## QUESTION 18–10

Why do you think apoptosis occurs by a different mechanism from the cell death that occurs in cell necrosis? What might be the consequences if apoptosis were not achieved in so neat and orderly a fashion, whereby the cell destroys itself from within and avoids leakage of its contents into the extracellular space?

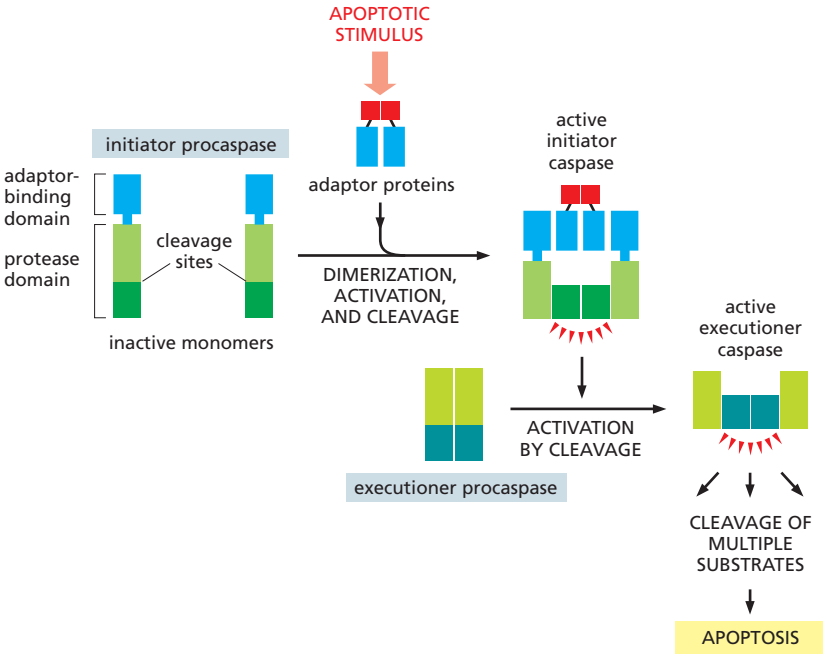



**Figure 18–39 Apoptosis is mediated by an intracellular proteolytic cascade.** An initiator caspase is first made as an inactive monomer called a procaspase. An apoptotic signal triggers the assembly of adaptor proteins that bring together a pair of initiator caspases, which are thereby activated, leading to cleavage of a specific site in their protease domains. Executioner caspases are initially formed as inactive dimers. Upon cleavage by an initiator caspase, the executioner caspase dimer undergoes an activating conformational change. The executioner caspases then cleave a variety of key proteins, leading to apoptosis.

In this way, the cell dismantles itself quickly and cleanly, and its corpse is rapidly taken up and digested by another cell.

Activation of the apoptotic program, like entry into a new stage of the cell cycle, is usually triggered in an all-or-none fashion: once a cell reaches a critical point along the path to destruction, it cannot turn back.

## The Intrinsic Apoptotic Death Program Is Regulated by the Bcl2 Family of Intracellular Proteins

All nucleated animal cells contain the seeds of their own destruction: in these cells, inactive procaspases lie waiting for a signal to destroy the cell. It is therefore not surprising that caspase activity is tightly regulated to ensure that the death program is held in check until it is needed—for example, to eliminate cells that are superfluous, mislocated, or badly damaged.

The main proteins that regulate the activation of caspases are members of the **Bcl2 family** of intracellular proteins. Some members of this protein family promote caspase activation and cell death, whereas others inhibit these processes. Two of the most important death-inducing family members are proteins called *Bax* and *Bak*. These proteins—which are activated in response to DNA damage or other insults—promote cell death by inducing the release of the electron-transport protein cytochrome *c* from mitochondria into the cytosol. Other members of the Bcl2 family (including Bcl2 itself) inhibit apoptosis by preventing Bax and Bak from releasing cytochrome *c*. The balance between the activities of pro-apoptotic and anti-apoptotic members of the Bcl2 family largely determines whether a cell lives or dies by apoptosis.

The cytochrome *c* molecules released from mitochondria activate initiator procaspases—and induce cell death—by promoting the assembly of a large, seven-armed, pinwheel-like protein complex called an apoptosome. The apoptosome then recruits and activates a particular initiator procaspase, which then triggers a caspase cascade that leads to apoptosis (**Figure 18–40**).

## Apoptotic Signals Can Also Come from Other Cells

Sometimes the signal to commit suicide is not generated internally, but instead comes from a neighboring cell. Some of these extracellular signals activate the cell death program by altering the activity of members of the Bcl2 family of proteins. Others stimulate apoptosis more directly by activating a set of cell-surface receptor proteins known as *death receptors*.

One particularly well-understood death receptor, called *Fas*, is present on the surface of a variety of mammalian cell types. Fas is activated by a membrane-bound protein, called *Fas ligand*, present on the surface of specialized immune cells called *killer lymphocytes*. These killer cells help regulate immune responses by inducing apoptosis in other immune cells that are unwanted or are no longer needed—and activating Fas is one way they do so. The binding of Fas ligand to its receptor triggers the assembly of a death-inducing signaling complex, which includes specific initiator procaspases that, when activated, launch a caspase cascade that leads to cell death.

## Animal Cells Require Extracellular Signals to Survive, Grow, and Divide

In a multicellular organism, the fate of individual cells is controlled by signals from other cells. Such communication ensures that a cell survives only when it is needed and divides only when another cell is required, either to allow tissue growth or to replace cell loss.

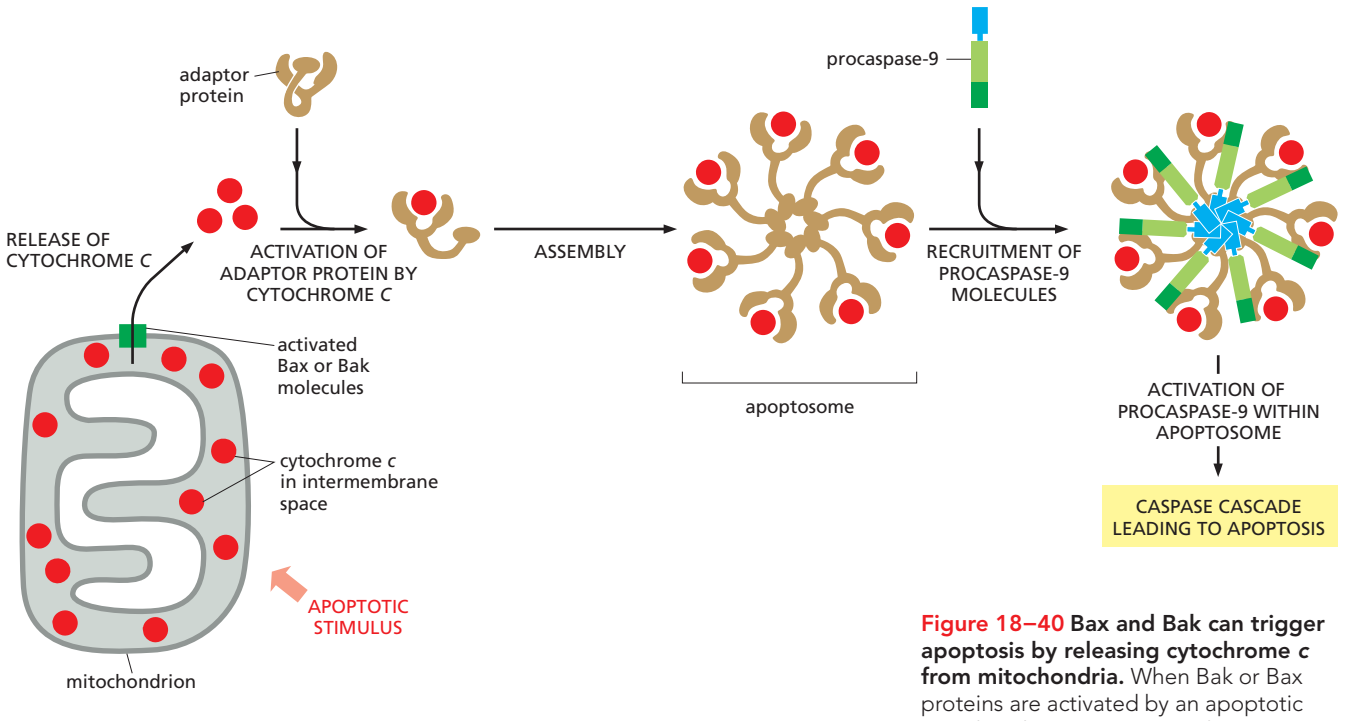



**Figure 18–40 Bax and Bak can trigger apoptosis by releasing cytochrome *c* from mitochondria.** When Bak or Bax proteins are activated by an apoptotic stimulus, they aggregate in the outer mitochondrial membrane, leading to the release of cytochrome *c* into the cytosol by an unknown mechanism. Additional proteins in the mitochondrial intermembrane space are released at the same time—not shown. Cytochrome *c* then binds to an adaptor protein, causing it to assemble into a seven-armed complex called the apoptosome. This complex then recruits seven molecules of a specific initiator procaspase (procaspase-9). The procaspase-9 proteins become activated within the apoptosome and then go on to activate executioner procaspases in the cytosol (as shown in Figure 18–39), leading to a caspase cascade and apoptosis.

Most of the extracellular signal molecules that influence cell survival, cell growth, and cell division are either soluble proteins secreted by other cells or proteins that are bound to the surface of other cells or to the extracellular matrix. Although most act positively to stimulate one or more of these cell processes, some act negatively to inhibit a particular process. The positively acting signal proteins can be classified, on the basis of their function, into three major categories:

1. **Survival factors** promote cell survival, largely by suppressing apoptosis.
2. **Mitogens** stimulate cell division, primarily by overcoming the intracellular braking mechanisms that block entry into the cell cycle in late $G_1$.
3. **Growth factors** stimulate cell growth (an increase in cell size and mass) by promoting the synthesis and inhibiting the degradation of proteins and other macromolecules.

These categories are not mutually exclusive, as many signal molecules have more than one of these functions. Unfortunately, the term "growth factor" is often used as a catch-all phrase to describe a protein with any of these functions. Indeed, the phrase "cell growth" is frequently used inappropriately to mean an increase in cell number, which is more correctly termed "cell proliferation."

In the following three sections, we examine each of these types of signal molecules in turn.

## Survival Factors Suppress Apoptosis

Animal cells need signals from other cells just to survive. If deprived of such survival factors, cells activate a caspase-dependent intracellular suicide program and die by apoptosis. This requirement for signals from other cells helps ensure that cells survive only when and where they are needed. Many types of nerve cells, for example, are produced in excess in the developing nervous system and then compete for limited amounts of survival factors that are secreted by the target cells they contact. Those

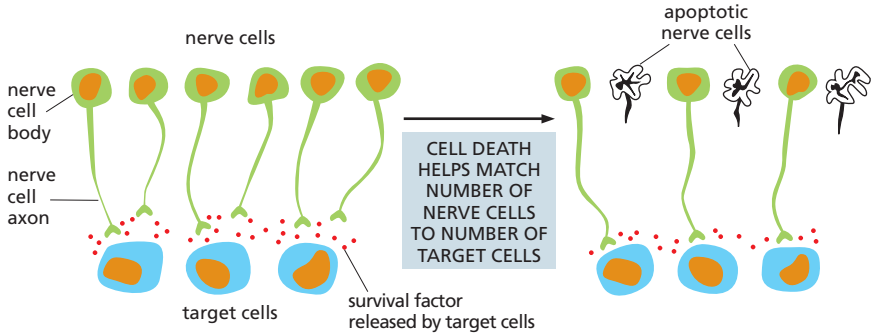



**Figure 18–41 Cell death can help adjust the number of developing nerve cells to the number of target cells they contact.** If more nerve cells are produced than can be supported by the limited amount of survival factor released by the target cells, some cells will receive insufficient amounts of survival factor to keep their suicide program suppressed and will undergo apoptosis. This strategy of overproduction followed by culling can help ensure that all target cells are contacted by nerve cells and that the "extra" nerve cells are automatically eliminated.

nerve cells that receive enough survival factor live, while the others die by apoptosis. In this way, the number of surviving nerve cells is automatically adjusted to match the number of cells with which they connect (**Figure 18–41**). A similar dependence on survival signals from neighboring cells is thought to help control cell numbers in other tissues, both during development and in adulthood.

Survival factors usually act through cell-surface receptors. Once activated, the receptors turn on intracellular signaling pathways that keep the apoptotic death program suppressed, usually by regulating members of the Bcl2 family of proteins. Some survival factors, for example, increase the production of Bcl2, a protein that suppresses apoptosis (**Figure 18–42**).

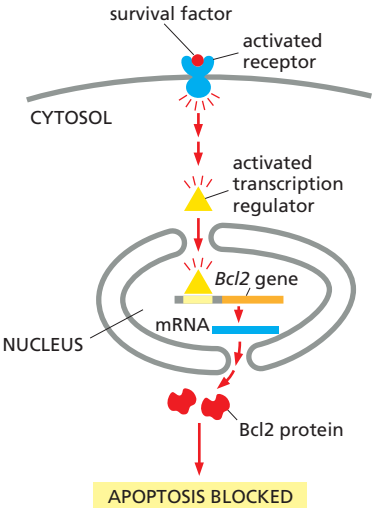



**Figure 18–42 Survival factors often suppress apoptosis by regulating Bcl2 family members.** In this case, the survival factor binds to cell-surface receptors that activate an intracellular signaling pathway, which in turn activates a transcription regulator in the cytosol. This protein moves to the nucleus, where it activates the gene encoding Bcl2, a protein that inhibits apoptosis (see also Figure 16–33).

## Mitogens Stimulate Cell Division by Promoting Entry into S Phase

Most mitogens are secreted signal proteins that bind to cell-surface receptors. When activated by mitogen binding, these receptors initiate various intracellular signaling pathways (discussed in Chapter 16) that stimulate cell division. As we saw earlier, these signaling pathways act mainly by releasing the molecular brakes that block the transition from the $G_1$ phase of the cell cycle into S phase (see Figure 18–14).

Most mitogens have been identified and characterized by their effects on cells in culture. One of the first mitogens identified in this way was *platelet-derived growth factor*, or *PDGF*, the effects of which are typical of many others discovered since. When blood clots form (in a wound, for example), blood platelets incorporated in the clots are stimulated to release PDGF. PDGF then binds to receptor tyrosine kinases (discussed in Chapter 16) in surviving cells at the wound site, stimulating these cells to proliferate and help heal the wound. In a similar way, if part of the liver is lost through surgery or acute injury, a mitogen called *hepatocyte growth factor* helps stimulate the surviving liver cells to proliferate.

## Growth Factors Stimulate Cells to Grow

The growth of an organ—or an entire organism—depends as much on cell growth as it does on cell division. If cells divided without growing, they would get progressively smaller, and there would be no increase in total cell mass. In single-celled organisms such as yeasts, both cell growth and cell division require only nutrients. In animals, by contrast, both cell growth and cell division depend on signals from other cells. Cell growth, unlike cell division, does not depend on the cell-cycle control system. Indeed, many animal cells, including nerve cells and most muscle cells, do most of their growing after they have terminally differentiated and permanently stopped dividing.

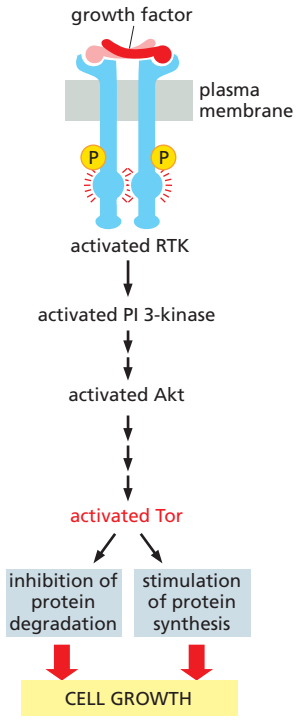



**Figure 18–43 Extracellular growth factors increase the synthesis and decrease the degradation of macromolecules.** Binding of a growth factor to a receptor tyrosine kinase (RTK, a class of cell-surface receptor described in Chapter 16) initiates an intracellular signaling pathway that leads to activation of a protein kinase called Tor, which acts through multiple targets to stimulate protein synthesis and inhibit protein degradation (see also Figure 16–34). This action leads to a net increase in macromolecules and thereby cell growth.

Like most survival factors and mitogens, most extracellular growth factors bind to cell-surface receptors that activate intracellular signaling pathways. These pathways lead to the accumulation of proteins and other macromolecules. Growth factors both increase the rate of synthesis of these molecules and decrease their rate of degradation (**Figure 18–43**).

Some extracellular signal proteins, including PDGF, can act as both growth factors and mitogens, stimulating both cell growth and progression through the cell cycle. Such proteins help ensure that cells maintain their appropriate size as they proliferate.

Compared to cell division, there has been surprisingly little study of how cell size is controlled in animals. As a result, it remains a mystery how different cell types in the same animal grow to be so different in size (**Figure 18–44**).

## Some Extracellular Signal Proteins Inhibit Cell Survival, Division, or Growth

The extracellular signal proteins that promote survival, growth, and cell division act positively to increase the size of organs and organisms. Some extracellular signal proteins, however, act to oppose these positive regulators and thereby inhibit tissue growth. *Myostatin*, for example, is a secreted signal protein that normally inhibits the growth and proliferation of the precursor cells (myoblasts) that fuse to form skeletal muscle cells during mammalian development. When the gene that encodes myostatin is deleted in mice, their muscles grow to be several times larger than normal, because both the number and the size of muscle cells is increased. Remarkably, two breeds of cattle that were bred for large muscles turned out to have mutations in the gene encoding myostatin (**Figure 18–45**).

Cancers are similarly the products of mutations that set cells free from the normal "social" controls operating on cell survival, growth, and proliferation. Because cancer cells are generally less dependent than normal cells on signals from other cells, they can out-survive, outgrow, and outdivide their normal neighbors, producing tumors that can kill their host (see Chapter 20).

In our discussions of cell division, we have focused entirely on the ordinary divisions that produce two daughter cells, each with a full and identical complement of the parent cell's genetic material. There is, however, a different and highly specialized type of cell division called meiosis, which is required for sexual reproduction in eukaryotes. In the next chapter, we describe the special features of meiosis and how they underlie the genetic principles that define the laws of inheritance.


**Figure 18–44 The cells in an animal can differ greatly in size.** The neuron and liver cell shown here are drawn at the same scale. A neuron grows progressively larger after it has terminally differentiated and permanently stopped dividing. (Neuron adapted from S. Ramón y Cajal, Histologie du Système Nerveux de l'Homme et de Vertébrés, 1909–1911. Paris: Maloine; reprinted, Madrid: C.S.I.C., 1972.)

(A) (B)

**Figure 18–45 Mutation of the *myostatin* gene leads to a dramatic increase in muscle mass.** (A) This Belgian Blue was produced by cattle breeders and was only later found to have a mutation in the *myostatin* gene. (B) Mice purposely made deficient in the same gene also have remarkably big muscles. A normal mouse is shown at the top for comparison with the muscular mutant shown at the bottom. (A, Yann Arthus-Bertrand/Getty Images; B, from S.-J. Lee, *PLoS One* 2:e789, 2007.)

## ESSENTIAL CONCEPTS

- The eukaryotic cell cycle consists of several distinct phases. In interphase, the cell grows and the nuclear DNA is replicated; in M phase, the nucleus divides (mitosis) followed by the cytoplasm (cytokinesis).
- In most cells, interphase consists of an S phase when DNA is duplicated plus two gap phases: $G_1$ and $G_2$. These gap phases give proliferating cells more time to grow and prepare for S phase and M phase.
- The cell-cycle control system coordinates events of the cell cycle by sequentially and cyclically switching on and off the appropriate parts of the cell-cycle machinery.
- The cell-cycle control system depends on cyclin-dependent protein kinases (Cdks), which are cyclically activated by the binding of cyclin proteins and by phosphorylation and dephosphorylation; when activated, Cdks phosphorylate key proteins in the cell.
- Different cyclin–Cdk complexes trigger different steps of the cell cycle: $G_1$-Cdk drives the cell through $G_1$; $G_1$/S-Cdk and S-Cdk drive it into S phase; and M-Cdk drives it into mitosis.
- The control system also uses protein complexes, such as APC/C, to trigger the destruction of specific cell-cycle regulators at particular stages of the cycle.
- The cell-cycle control system can halt the cycle at specific transition points to ensure that intracellular and extracellular conditions are favorable and that each step is completed before the next is started. Some of these control mechanisms rely on Cdk inhibitors that block the activity of one or more cyclin–Cdk complexes.
- S-Cdk initiates DNA replication during S phase and helps ensure that the genome is copied only once. The cell-cycle control system can delay cell-cycle progression during $G_1$ or S phase to prevent cells from replicating damaged DNA. It can also delay the start of M phase to ensure that DNA replication is complete.
- Centrosomes duplicate during S phase and separate during $G_2$. Some of the microtubules that grow out of the duplicated centrosomes interact to form the mitotic spindle.

- When the nuclear envelope breaks down, the spindle microtubules capture the duplicated chromosomes and pull them in opposite directions, positioning the chromosomes at the equator of the metaphase spindle.
- The sudden separation of sister chromatids at anaphase allows the chromosomes to be pulled to opposite poles; this movement is driven by the depolymerization of spindle microtubules and by microtubule-associated motor proteins.
- A nuclear envelope re-forms around the two sets of segregated chromosomes to form two new nuclei, thereby completing mitosis.
- In animal cells, cytokinesis is mediated by a contractile ring of actin filaments and myosin filaments, which assembles midway between the spindle poles; in plant cells, by contrast, a new cell wall forms inside the parent cell to divide the cytoplasm in two.
- In animals, extracellular signals regulate cell numbers by controlling cell survival, cell growth, and cell proliferation.
- Most animal cells require survival signals from other cells to avoid apoptosis—a form of cell suicide mediated by a proteolytic caspase cascade; this strategy helps ensure that cells survive only when and where they are needed.
- Animal cells proliferate only if stimulated by extracellular mitogens produced by other cells; mitogens release the normal intracellular brakes that block progression from $G_1$ or $G_0$ into S phase.
- For an organism or an organ to grow, cells must grow as well as divide; animal cell growth depends on extracellular growth factors that stimulate protein synthesis and inhibit protein degradation.
- Some extracellular signal molecules inhibit rather than promote cell survival, cell growth, or cell division.
- Cancer cells fail to obey these normal "social" controls on cell behavior and therefore outgrow, out-divide, and out-survive their normal neighbors.

## KEY TERMS

anaphase
anaphase-promoting complex (APC/C)
apoptosis
aster
Bcl2 family
bi-orientation
caspase
Cdk (cyclin-dependent protein kinase)
Cdk inhibitor protein
cell cycle
cell-cycle control system
centrosome
centrosome cycle
chromosome condensation
cohesin
condensin
contractile ring
cyclin
cytokinesis
$G_1$-Cdk
$G_1$ cyclin
$G_1$ phase
$G_2$ phase
$G_1$/S-Cdk
$G_1$/S cyclin
growth factor
interphase
kinetochore
M-Cdk
M cyclin
M phase
metaphase
mitogen
mitosis
mitotic spindle
p53
phragmoplast
programmed cell death
prometaphase
prophase
S-Cdk
S cyclin
S phase
sister chromatid
spindle pole
survival factor
telophase

# QUESTIONS

## QUESTION 18–11

Roughly, how long would it take a single fertilized human egg to make a cluster of cells weighing 70 kg by repeated divisions, if each cell weighs 1 nanogram just after cell division and each cell cycle takes 24 hours? Why does it take very much longer than this to make a 70 kg adult human?

## QUESTION 18–12

The shortest eukaryotic cell cycles of all—shorter even than those of many bacteria—occur in many early animal embryos. These so-called cleavage divisions take place without any significant increase in the weight of the embryo. How can this be? Which phase of the cell cycle would you expect to be most reduced?

## QUESTION 18–13

One important biological effect of a large dose of ionizing radiation is to halt cell division.

A. How does this occur?

B. What happens if a cell has a mutation that prevents it from halting cell division after being irradiated?

C. What might be the effects of such a mutation if the cell is not irradiated?

D. An adult human who has reached maturity will die within a few days of receiving a radiation dose large enough to stop cell division. What does that tell you (other than that one should avoid large doses of radiation)?

## QUESTION 18–14

If cells are grown in a culture medium containing radioactive thymidine, the thymidine will be covalently incorporated into the cell's DNA during S phase. The radioactive DNA can be detected in the nuclei of individual cells by autoradiography: radioactive cells will activate a photographic emulsion and be labeled by black dots when looked at under a microscope. Consider a simple experiment in which cells are radioactively labeled by this method for only a short period (about 30 minutes). The radioactive thymidine medium is then replaced with one containing unlabeled thymidine, and the cells are grown for some additional time. At different time points after replacement of the medium, cells are examined in a microscope. The fraction of cells in mitosis (which can be easily recognized because the cells have rounded up and their chromosomes are condensed) that have radioactive DNA in their nuclei is then determined and plotted as a function of time after the labeling with radioactive thymidine (Figure Q18–14).

A. Would all cells (including cells at all phases of the cell cycle) be expected to contain radioactive DNA after the labeling procedure?

B. Initially, there are no mitotic cells that contain radioactive DNA (see Figure Q18–14). Why is this?

C. Explain the rise and fall and then rise again of the curve.

D. Estimate the length of the $G_2$ phase from this graph.

## QUESTION 18–15

One of the functions of M-Cdk is to cause a precipitous drop in M cyclin concentration halfway through M phase. Describe the consequences of this sudden decrease and suggest possible mechanisms by which it might occur.

## QUESTION 18–16

Figure 18–5 shows the rise of M cyclin concentration and the rise of M-Cdk activity in cells as they progress through the cell cycle. It is remarkable that the M cyclin concentration rises slowly and steadily, whereas M-Cdk activity increases suddenly. How do you think this difference arises?

## QUESTION 18–17

What is the order in which the following events occur during cell division?

A. anaphase

B. metaphase

C. prometaphase

D. telophase

E. mitosis

F. prophase

Where does cytokinesis fit in?

## QUESTION 18–18

The lifetime of a microtubule in a mammalian cell, between its formation by polymerization and its spontaneous disappearance by depolymerization, varies with the stage of the cell cycle. For an actively proliferating cell, the average lifetime is 5 minutes in interphase and 15 seconds in mitosis. If the average length of a microtubule in interphase is 20 μm, how long will it be during mitosis, assuming that the rates of microtubule elongation due to the addition of tubulin subunits in the two phases are the same?

## QUESTION 18–19

The balance between plus-end directed and minus-end directed motor proteins that bind to interpolar microtubules in the overlap region of the mitotic spindle is thought to

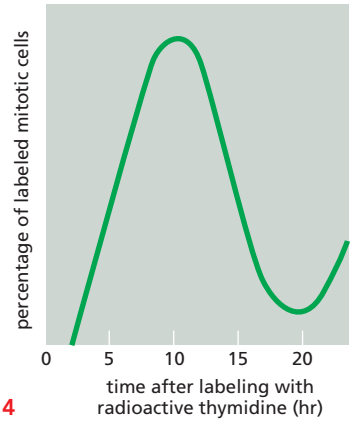



Figure Q18–14

help determine the length of the spindle. How might each type of motor protein contribute to the determination of spindle length?

### QUESTION 18–20

Sketch the principal stages of mitosis, using Panel 18–1 (pp. 628–629) as a guide. Color one sister chromatid and follow it through mitosis and cytokinesis. What event commits this chromatid to a particular daughter cell? Once initially committed, can its fate be reversed? What may influence this commitment?

### QUESTION 18–21

The polar movement of chromosomes during anaphase A is associated with microtubule shortening. In particular, microtubules depolymerize at the ends at which they are attached to the kinetochores. Sketch a model that explains how a microtubule can shorten and generate force yet remain firmly attached to the chromosome.

### QUESTION 18–22

Rarely, both sister chromatids of a replicated chromosome end up in one daughter cell. How might this happen? What could be the consequences of such a mitotic error?

### QUESTION 18–23

Which of the following statements are correct? Explain your answers.

A. Centrosomes are replicated before M phase begins.

B. Two sister chromatids arise by replication of the DNA of the same chromosome and remain paired as they line up on the metaphase plate.

C. Interpolar microtubules attach end-to-end and are therefore continuous from one spindle pole to the other.

D. Microtubule polymerization and depolymerization and microtubule motor proteins are all required for DNA replication.

E. Microtubules nucleate at the centromeres and then connect to the kinetochores, which are structures at the centrosome regions of chromosomes.

### QUESTION 18–24

An antibody that binds to myosin prevents the movement of myosin molecules along actin filaments (the interaction between actin and myosin is described in Chapter 17). How do you suppose the antibody exerts this effect? What might be the result of injecting this antibody into cells (A) on the movement of chromosomes at anaphase or (B) on cytokinesis? Explain your answers.

### QUESTION 18–25

Look carefully at the electron micrographs in Figure 18–38. Describe the differences between the cell that died by necrosis and those that died by apoptosis. How do the pictures confirm the differences between the two processes? Explain your answer.

### QUESTION 18–26

Which of the following statements are correct? Explain your answers.

A. Cells do not pass from $G_1$ into M phase of the cell cycle unless there are sufficient nutrients to complete an entire cell cycle.

B. Apoptosis is mediated by special intracellular proteases, one of which cleaves nuclear lamins.

C. Developing neurons compete for limited amounts of survival factors.

D. Some vertebrate cell-cycle control proteins function when expressed in yeast cells.

E. The enzymatic activity of a Cdk protein is determined both by the presence of a bound cyclin and by the phosphorylation state of the Cdk.

### QUESTION 18–27

Compare the rules of cell behavior in an animal with the rules that govern human behavior in society. What would happen to an animal if its cells behaved as people normally behave in our society? Could the rules that govern cell behavior be applied to human societies?

### QUESTION 18–28

In his highly classified research laboratory, Dr. Lawrence M. is charged with the task of developing a strain of dog-sized rats to be deployed behind enemy lines. In your opinion, which of the following strategies should Dr. M. pursue to increase the size of rats?

A. Block all apoptosis.

B. Block p53 function.

C. Overproduce growth factors, mitogens, or survival factors.

Explain the likely consequences of each option.

### QUESTION 18–29

PDGF is encoded by a gene that can cause cancer when expressed inappropriately. Why do cancers not arise at wounds in which PDGF is released from platelets?

### QUESTION 18–30

What do you suppose happens in mutant cells that

A. cannot degrade M cyclin?

B. always express high levels of p21?

C. cannot phosphorylate Rb?

### QUESTION 18–31

Liver cells proliferate excessively both in patients with chronic alcoholism and in patients with liver cancer. What are the differences in the mechanisms by which cell proliferation is induced in these diseases?