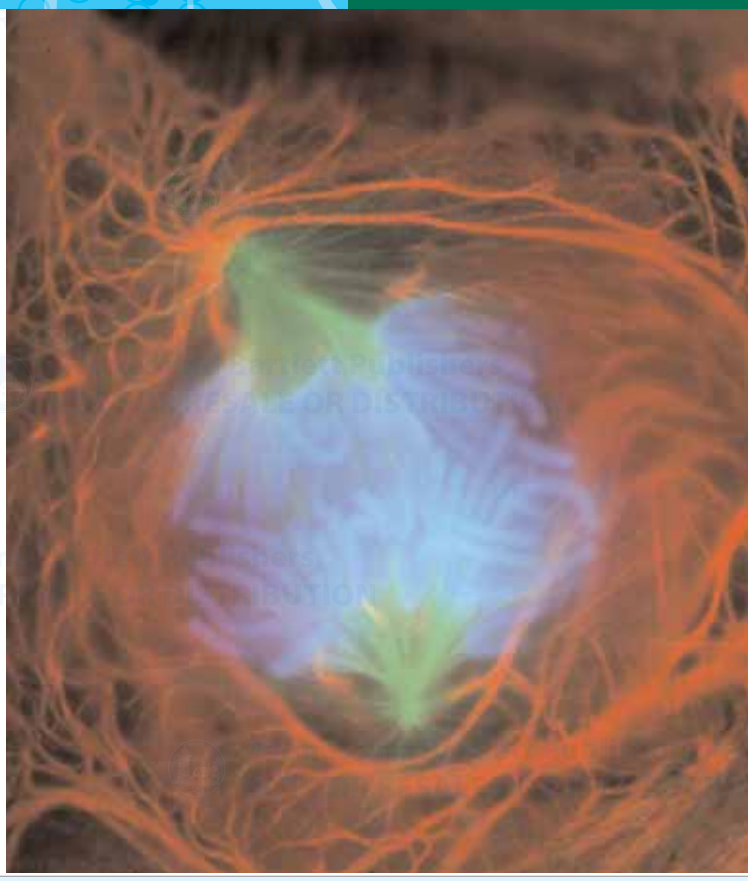
10

Mitosis



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This fluorescence micrograph shows the anaphase stage of cell division in a salamander lung cell. During this stage the genes, which are contained within the chromosomes, are equally segregated into two well-separated daughter nuclei. Photo © Conly Rieder, Wadsworth Center.

CHAPTER OUTLINE

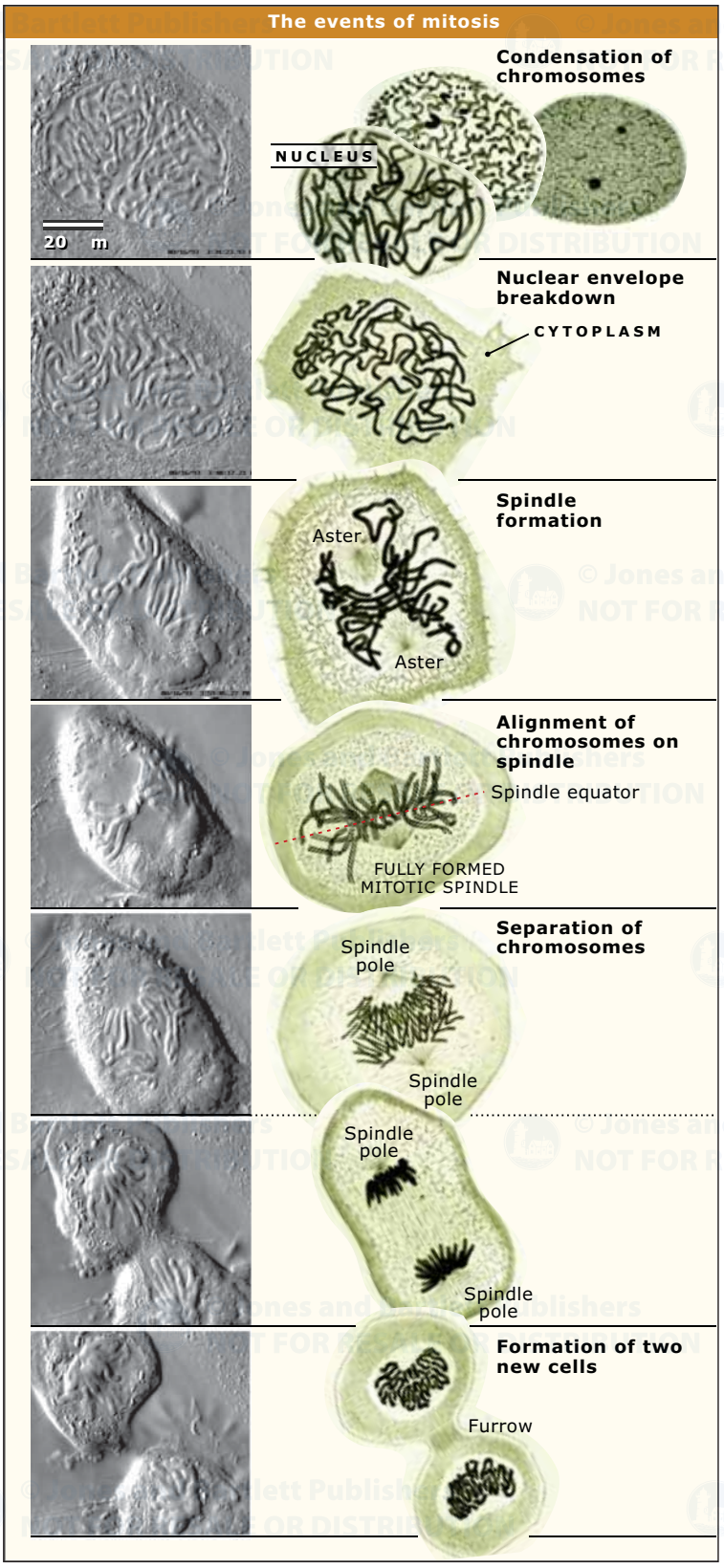

FIGURE 10.1 In the top panel, only the nucleus is shown. The remaining panels show the entire cell. After spindle formation, the two spindle poles are located in the center of the clear areas in the cytoplasm at the upper left and lower right of the cell. Photos © Conly Rieder, Wadsworth Center. Illustrations from Flemming, W., 1879. *Archiv fur Mikroskopische Anatomie*.

10.1 Introduction

Key concepts

- All cells are produced by the division of other cells through a process called mitosis.
- Mitosis occurs after a cell has replicated its chromosomes. Mitosis separates the chromosomes into two equal groups and then divides the cell between them to form two new cells.
- Errors in mitosis are catastrophic, and mechanisms have evolved to ensure its accuracy.

Perhaps the most fundamental activity of cells is reproduction: life depends on the ability of cells to divide. In single-celled organisms, to divide is to reproduce. In complex multicellular organisms, division is required not only to produce the cells essential for development and growth but also to replace cells as they die.

The term *cell* was coined in 1665 by Robert Hooke, who used it to describe the hollow cubicles seen in thin slices of cork when viewed under a microscope. It took 175 years of further microscopic observations before Schleiden and Schwann recognized with their *Cell Theory* that cells are the fundamental building blocks of life. As this major landmark of nineteenth-century science gained general acceptance, a logical next question was: How are new cells formed? Although some people believed that new cells arose spontaneously, in 1855 the German physician Virchow made the definitive argument *omnis cellula e cellula*—that every cell is the offspring of a preexisting parent cell.

With the invention and widespread use of the compound light microscope in the late nineteenth century, progress in describing the events that take place as a cell divides accelerated rapidly. In 1879, the German anatomist Walther Flemming used the term mitosis to characterize the formation of what resembled paired threads (Greek: *mitos* = threads) inside the nucleus of dividing salamander cells, and he described a series of changes that they underwent, shown in FIGURE 10.1. These threads, which formed from a substance within the nucleus that Flemming called chromatin, came to be known as the chromosomes (Greek: *chroma* = color; *soma* = body). Flemming noted that during the early stages of mitosis, every chromosome consisted of two identical threads, or chromatids, that are stuck to one another along their length, as shown in FIGURE 10.2. In higher organisms, every chromosome contains a small but conspicuous region at which it narrows, known as the primary constriction or cen-

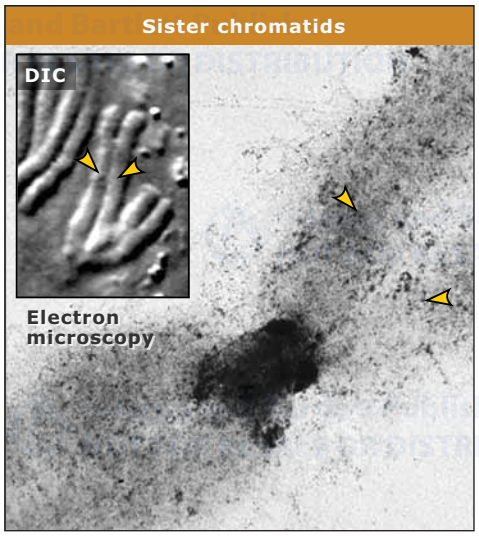

FIGURE 10.2 The inset shows an entire metaphase chromosome in a living newt cell. The larger photograph shows the region around the primary constriction of another metaphase chromosome. In both photographs the arrowheads indicate the paired sister chromatids. (DIC: differential interference microscopy, a form of light microscopy). Photo courtesy of Jerome B. Rattner, University of Calgary, Canada (main), and photo © Conly Rieder, Wadsworth Center (inset).

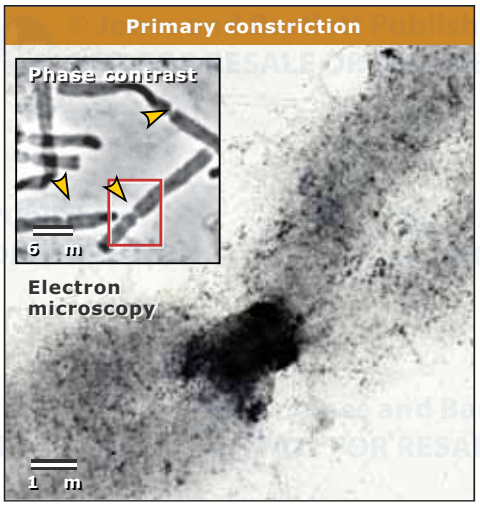

FIGURE 10.3 The inset shows several entire metaphase chromosomes in a live newt cell. Each narrows at a unique point called the primary constriction. The electron micrograph shows a highly magnified view of the primary constriction of a single chromosome. Photo courtesy of Jerome B. Rattner, University of Calgary, Canada (main), and photo © Conly Rieder, Wadsworth Center (inset).

tromere, as shown in **FIGURE 10.3**. Every cell within an organism has the same number of chromosomes, which is the same for all the members of a species. The number of chromosomes per cell differs among species, however—some having many times more chromosomes than others.

As early as 1880, Flemming argued that all cells reproduce through the "metamorphosis of the nuclear mass into threads." By 1883 observations on the fertilization of sea urchin eggs proved that the egg and sperm contribute an equal number of chromosomes to the embryo. Two years later it was shown that all the nuclei in an organism are generated by repeated divisions of the single nucleus formed within the embryo from a fusion between the egg and sperm nuclei. Thus, by 1885 it was evident that every cell contains chromosomes from both parents. This conclusion connected the *Cell Theory* (1838) of Schleiden and Schwann with Darwin's *Theory of Evolution* (1859). The nature of this connection was later established with the discovery that the chromosomes contain a cell's genes, the units that transmit properties between generations.

With the exception of sperm and eggs, all of the cells in the body are diploid (di = 2), in that two copies of each chromosome are present: one inherited from the mother via the egg and the other from the father via the sperm. (Human cells contain 23 pairs of chromosomes, giving humans a total of 46 chromosomes.) *The purpose of mitosis is to preserve the diploid number of chromosomes over repeated generations of cells.* Since sperm and eggs are haploid, in that they contain only half the number of chromosomes found in tissue cells, they cannot be produced by mitosis. Rather, these specialized cells (called gametes) are formed by a process known as meiosis, as shown in **FIGURE 10.4**. During meiosis four haploid cells, each containing just one copy of every chromosome, are produced from one precursor cell. This reduction in chromosome number results from dividing the cell twice after its chromosomes have been replicated, rather than just once as in mitosis. Unlike mitosis, the purpose of meiosis is to maintain the diploid number of chromosomes *over repeated generations of the organism*. In practice, mitosis and meiosis involve many of the same mechanisms—the major difference being how the chromosomes are organized at the start of the process.

This chapter will focus on how mitosis works in higher animals, specifically vertebrates. Although the details can vary depending on the

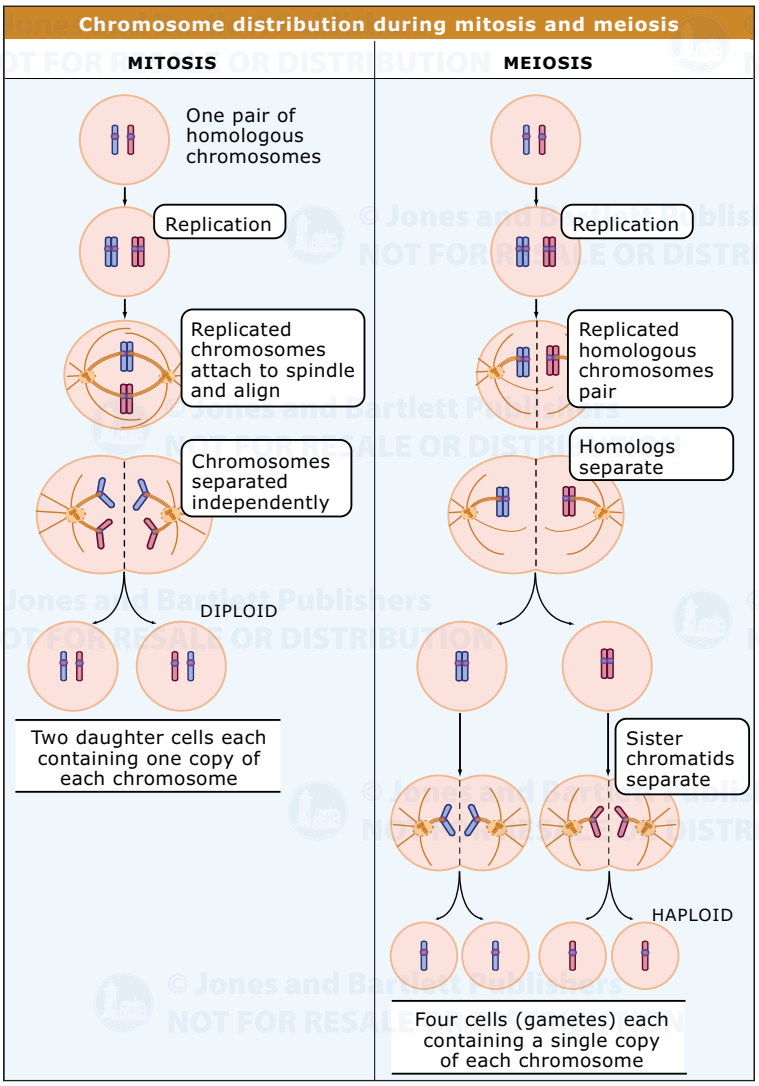

FIGURE 10.4 Meiosis involves two cell divisions in sequence. The first separates homologous chromosomes, the second the individual chromatids of each chromosome. In mitosis only the chromatids are separated.

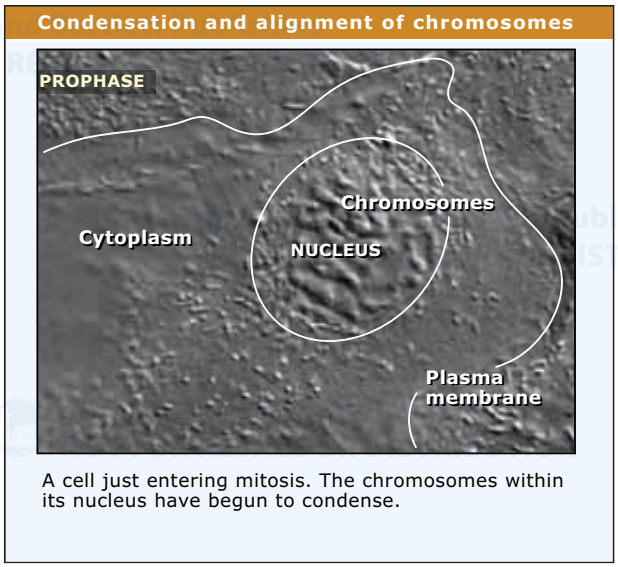

FIGURE 10.5 The first frames of a video (see CBIO: 10-0001), which follows the chromosomes through the intial stages of mitosis. Photo © Conly Rieder and Alexey Khodjakov, Wadsworth Center.

organism, the fundamental aspects of mitosis are similar in all cells. The stages can be seen in Figure 10.1. In higher animals, the first visible sign of an impending division is the appearance of the replicated chromosomes within the nucleus. Once this condensation of chromosomes is well underway, the envelope surrounding the nucleus disintegrates, dispersing the chromosomes into the cytoplasm. Next, the chromosomes become attached to a structure called the spindle, named because it is shaped like two cones joined at their wide ends. This spindle, or mitotic apparatus, is responsible for generating the forces for moving the chromosomes and also for directing where in the cell they will move. Once attached, the chromosomes gradually become aligned across the middle of the spindle, which is referred to as its equator. A video, whose first frame is shown in FIGURE 10.5, shows the entire sequence of events from chromosome condensation to alignment.

After all the chromosomes are aligned, each splits lengthwise (i.e., the chromatids separate), and the two independent groups of chromosomes that result move away from each other toward the opposite ends of the spindle, called the spindle poles. Finally, the chromosomes within each of the two separating groups decondense and a new envelope is formed around each. The multiple small nuclei formed at each end of the cell fuse together, giving rise to two independent daughter nuclei. The definition of mitosis has been expanded over the years to also include cytokinesis, the series of events by which the cell cytoplasm is partitioned after the nucleus has divided (see Figure 10.1).

Even though chromosome segregation occurs with a high level of accuracy, errors do occasionally occur. Mistakes during mitosis or meiosis can arise at several stages of the processes and can lead directly to cells that contain too few or too many chromosomes. *This condition is known as* aneuploidy, *and its consequences vary depending on the organism and the time when the mistake occurs.* When it arises during the production of gametes (meiosis), it will lead to an embryo with a birth defect syndrome in which all of its cells have at least one extra or missing chromosome. An example of aneuploidy in humans is Down's syndrome, in which all of the cells of an individual contain an extra copy of chromosome 21, as shown in FIGURE 10.6. In

FIGURE 10.6 The mitotic chromosomes from a single cell of a human with Down's syndrome. The different chromosomes can be distinguished by the position of their primary constriction, their size, and the pattern of dark and light bands in each. There are three copies of the small chromosome 21, but only two of each of the other chromosomes. Photo courtesy of Ann Wiley, Wadsworth Center.

most cases, however, aneuploidy in the embryo leads to death before development is complete. By contrast, when aneuploidy arises during development, a mosaic organism is formed, in which different tissues consist of cells containing different numbers of chromosomes. Finally, there is good evidence that the formation of aneuploid cells in adult organisms plays a role in initiating some cancers.

Because the equal distribution of the chromosomes is essential to an organism's viability, mitosis includes processes that are devoted solely to enhancing its accuracy. In all organisms, the accuracy of chromosome segregation is increased by checkpoint controls. Checkpoints are biochemical pathways that stop or delay division until a specific event is completed or corrected. The need for great accuracy is also reflected by the existence of multiple pathways for accomplishing the same goal, be it forming the spindle or moving the chromosomes. Although mitosis always proceeds through the sequence of events just described, several different possible routes are present to complete the more critical processes. This duplication of mechanisms, which has only recently been recognized, adds an extra layer of complexity to the mitotic process but gives it a flexibility that allows it to withstand conditions that would otherwise result in errors.

10.2 Mitosis is divided into stages

Key concepts

- Mitosis proceeds through a series of stages that are characterized by the location and behavior of the chromosomes.
- Some of the conversions between stages correspond to cell cycle events and are irreversible transitions.

Mitosis occurs through the initiation and completion of two separate and distinct processes. In the first, which is sometimes termed karyokinesis (Greek: *karyo* = nut; *kinesis* = division), the replicated chromosomes are separated into two distinct daughter nuclei. During the second process, called cytokinesis, the cytoplasm is divided between these two nuclei to form two independent daughter cells. Historically, the division of the nucleus is broken down into several stages that are defined by the structure and position of the chromosomes. Breaking down a complex series of events like mitosis into stages is useful because some transitions mark irreversible changes that have occurred within the cell. Most of these changes depend on the activation or inactivation of particular enzymes and sometimes also require the timely destruction of specific proteins that play strategic roles during division. Viewing mitosis as a series of stages is also useful because the chromosomes and the spindle both alter their behavior between stages, suggesting that each stage is characterized by specific mechanisms at the molecular level. As we discuss the stages in detail, refer to Figure 10.1.

The first visible sign of an impending division is the appearance of condensing chromosomes within the nucleus. This starts the prophase stage of mitosis. In cold-blooded animals that contain large chromosomes (e.g., salamanders, grasshoppers), this stage takes several hours; in warm-blooded creatures with small chromosomes (e.g., mice, humans), it may last less than 15 minutes. At some point in prophase, biochemical changes occur within the cell that commit it to mitosis. Before this point of no return is reached, chromosome condensation can be reversed by physical or chemical insults that damage the cell.

Prophase is also commonly marked by the appearance of the centrosomes. In many prophase cells, two of these organelles become visible in the cytoplasm as small dots surrounded by a clear area. As we will see, these centrosomes play an important role in spindle formation: not only will they define the two poles of the spindle; they will also nucleate the microtubules used in its construction.

Cells are driven into mitosis by the addition of phosphate groups to some proteins and their removal from others. Enzymes known as kinases and phosphatases accomplish this phosphorylation and dephosphorylation. During mitosis, the most important kinase is the cyclin B/CDK1 complex. This enzyme is considered to be the master mitotic regulator because when it is injected into cells mitosis is induced. (The discovery of this kinase and the mechanisms by which it is regulated was the subject of the 2001 Nobel Prize in Physiology and Medicine.) Near the end of prophase, cyclin B/CDK1 accumulates within the nucleus in an inactive form. Shortly thereafter, another enzyme, the cdc25 phosphatase, enters the nucleus, where it activates cyclin B/CDK1. Once activated, cyclin B/CDK1 phosphorylates many nuclear proteins, including those that provide structural support for the membrane surrounding the nucleus. As a result, these proteins lose their association with the nuclear membrane, causing the nucleus to swell until its surrounding membrane envelope disintegrates (see Figure 10.1).

The breakdown of the nuclear envelope marks the beginning of the prometaphase stage of mitosis. During this stage, the chromosomes interact with the two centrosomes and their associated arrays of microtubules to form the spindle (see Figure 10.1). As the chromosomes become attached to the spindle, they go through a series of complex motions called congression. During congression, chromosomes move both toward and away from the spindle poles. Each chromosome moves independently of the others, moving first toward one pole, then toward the other, often reversing direction several times before the process is complete. Ultimately, these movements lead to the *congregation* of all the chromosomes into a plane, or "plate," at the spindle equator, halfway between the two poles. In most cells, prometaphase is the longest stage of mitosis, since it lasts until all of the chromosomes are positioned at the equator. This may take just a few minutes in embryos or up to several hours in highly flattened tissue cells.

Once all of the chromosomes are near the spindle equator, the cell is considered to be in metaphase (see Figure 10.1). Metaphase can last for different lengths of time, depending on the cell type. Surprisingly, the complex events that have brought the cell to this point are reversible. When the spindle in a metaphase or prometaphase cell is destroyed by treating it with drugs (e.g., colcemid or nocodazole) or other agents (e.g., cold or high pressure) that depolymerize microtubules, it reforms and the chromosomes repeat the congression process as soon as the treatment is stopped. Dissolution of the spindle in metaphase cells prevents the cell cycle from advancing and is often used to experimentally produce cells that are considered as being "locked in metaphase." These cells are actually in prometaphase since their condensed chromosomes are scattered throughout the cytoplasm. (For more on microtubules see *7 Microtubules*.)

Metaphase ends when the two sister chromatids of each chromosome separate, beginning the anaphase stage of mitosis (see Figure 10.1). Although each chromosome was replicated before mitosis, its two constituent chromatids normally only become visible as distinct units shortly before metaphase ends (see Figure 10.2). In movies the separation of chromatids seems to occur suddenly and simultaneously for all chromosomes; in reality it usually takes several minutes and occurs at a slightly different time for each. *The separation of chromatids at the beginning of anaphase marks another point-of-no-return in mitosis: it coincides with the destruction of "glue" proteins that hold the chromatids together and with the destruction of the master regulatory kinase that drives cells into mitosis.* (For details see *11 Cell cycle regulation*.) After the sister chromatids have separated, they move away from one another toward different poles of the spindle. This movement occurs by a combination of two different mechanisms. During anaphase A, the distance between each chromatid and the pole to which it is attached decreases. At the same time, the two spindle poles themselves move further apart, pulling their attached groups of chromosomes with them in a process known as spindle elongation, or anaphase B (see Figure 10.1). As the two groups of chromosomes move apart, the spindle begins to disassemble, and new structures known in animal cells as stem bodies (see Figure 10.55) form between them.

The final telophase (Greek: *telo* = end) stage of mitosis begins as the chromosomes start to reform nuclei near the poles (see Figure 10.1).

In cases in which neighboring anaphase chromosomes are not touching as telophase begins (as in large cells), each chromosome forms its own small nucleus. These then fuse to form a single, larger nucleus. During telophase, the events that will divide the cell in two also begin. Initially, a furrow forms around the surface of the cell in the same plane in which the chromosomes were aligned at metaphase. In this position the furrow is located midway between the two new nuclei and encircles the stem bodies (see Figure 10.1). Once formed, the furrow gradually constricts, dividing the cell into two roughly equal lobes in the process of cytokinesis. As the furrow constricts, the stem bodies are gathered together into a tight bundle called the midbody, the last structure that connects the two cells (see Figure 10.55 and Figure 10.56). The events of telophase require the inactivation of cyclin B/CDK1, and they signal that the cell is leaving the mitotic state.

Discussing mitosis as a series of stages and looking at still photographs of live or fixed cells may make it appear as a somewhat static, discontinuous process. However, in reality it is continuous and highly dynamic, something that can only be fully appreciated by viewing movies of dividing cells such as the one whose first frame is shown in FIGURE 10.7.

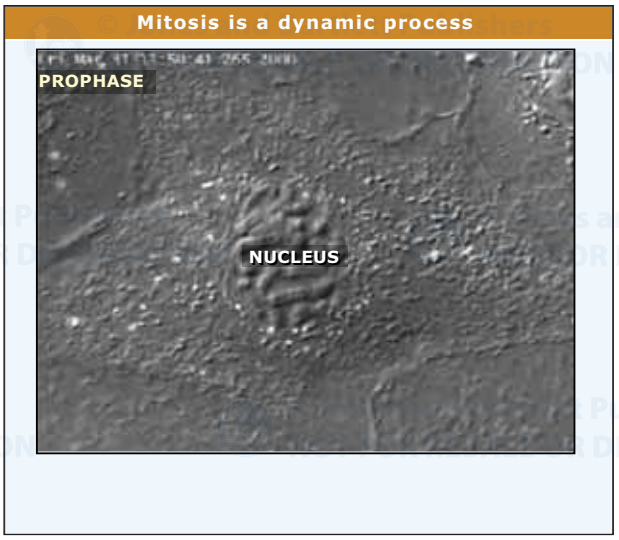

FIGURE 10.7 The first frame of a video (see CBIO: 10-0002), which shows mitosis from beginning to end. Photo © Conly Rieder and Alexey Khodjakov, Wadsworth Center.

10.3 Mitosis requires the formation of a new apparatus called the spindle

Key concepts

- The chromosomes are separated by the mitotic spindle.
- The spindle is a symmetrical, bipolar structure composed of microtubules that extend between two poles. At each pole is a centrosome.
- Chromosomes attach to the spindle via interactions between their kinetochores and the microtubules of the spindle.

The spindle is a dynamic and complex structure that suddenly appears as cell division begins and then quickly disassembles as the process is completed (see Figure 10.54). *The spindle is required for mitosis and serves two distinct functions: (1) it is responsible for separating the replicated chromosomes into daughter nuclei during division of the nucleus (karyokinesis), and (2) it directs the process of dividing the cytoplasm (cytokinesis).* When the spindle is prevented from forming (e.g., by treatment of the cell with various drugs), the chromosomes condense but do not undergo any of the movements of a normal mitosis and progression through division stops. In many ways, the spindle is a kind of biological machine that converts chemical energy into the mechanical work needed to move the chromosomes and divide the cell. Its function is reflected in its structure. The symmetrical structure of the spindle—with two poles—is essential for a successful mitosis. Indeed, it defines the inherent "two-ness" of cell division, in which one cell and its replicated DNA are divided equally into two separate daughter cells.

The spindle can be viewed by several means. Microtubules, the spindle's primary structural component, are too small to be seen with the light microscope (i.e., they cannot be resolved). As a result, although the condensed chromosomes can often be seen within the cells of higher animals by traditional forms of light microscopy, the spindle cannot. However, in many cells, the shape of the spindle can be inferred because it excludes most of the visible organelles from its volume. As FIGURE 10.8 shows, this causes the space occupied by the spindle to appear clear relative to the surrounding cytoplasm. Although scientists initially suspected that the spindle consisted of fibers, this idea was not proved until the early 1950s. At that time, refinements in polarization light microscopy allowed the spindle to be seen in living cells. A typical photograph of a spindle taken with this method is shown in Figure 10.8, where the spindle appears dark black because of the interaction between its mi-

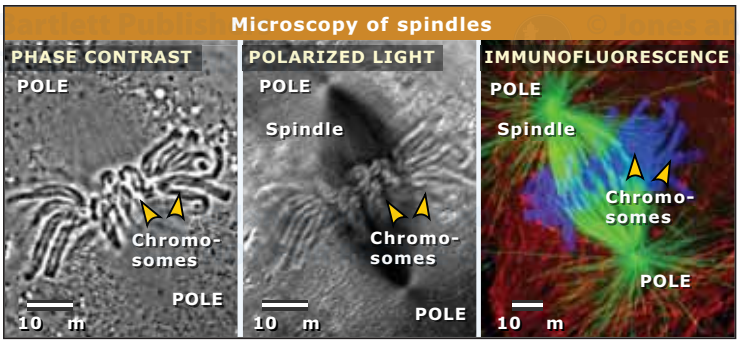

FIGURE 10.8 A metaphase spindle in a living newt cell viewed by phase contrast and polarized light microscopy. A similar cell with its spindle in the same orientation is shown in part after it was stained by immunofluorescence methods for microtubules (green), chromosomes (blue), and keratin filaments (red). Note that the spindle is invisible in the phase image but detected by polarized light. Spindle microtubules are most clearly seen after immunofluorescence staining. Reprinted with permission from Rieder, C., and A. Khodjakov. 2003. *Science*. 300: 91-96. © 2003 AAAS. (Left and center) Photos courtesy of Conly Rieder and Alexey Khodjakov, Wadsworth Center.

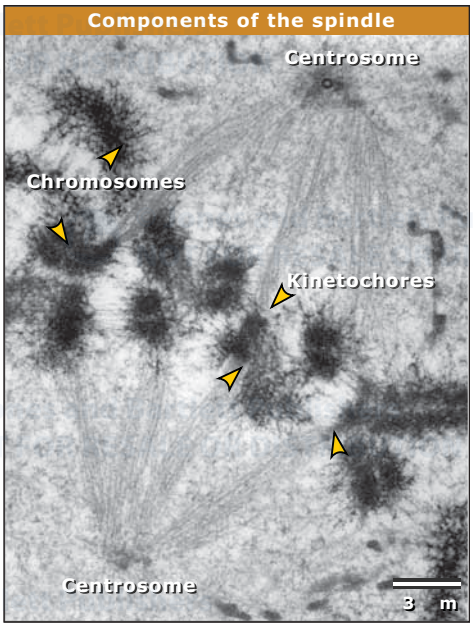

FIGURE 10.9 An electron micrograph showing the basic structural elements of a mitotic spindle. Large bundles of microtubules connect each centrosome to the kinetochores on the chromosomes. The labeled kinetochores in the center of the picture illustrate how the two kinetochores on a chromosome face opposite poles of the spindle. Reprinted with permission from Rieder, C., and A. Khodjakov. 2003. *Science.* 300: 91-96. © 2003 AAAS. Photo courtesy of Conly Rieder, Wadsworth Center.

crotubules and the polarized light. Since the 1970s, powerful fluorescence-tagging techniques have been developed that allow components of the spindle to be viewed in three dimensions, even in living cells (see Figure 10.8). With these techniques the positions of one or more specific proteins within the spindle can be determined and each followed over the course of mitosis. One of the proteins followed is almost always tubulin because it allows the microtubules to be visualized.

When viewed with the electron microscope, the mature animal cell spindle contains three primary structural components, as show in **FIGURE 10.9**. Each of the two polar areas is defined by a centrosome, as shown in **FIGURE 10.10**. This beautiful organelle consists of a pair of small, densely staining structures known as centrioles surrounded by a diffuse cloud of more lightly staining material. Positioned between the centrosomes are the chromosomes, which in most organisms are the largest structures in the spindle (see Figure 10.9). Chromosomes are composed of compacted, tightly coiled and heavily staining chromatin fibers 25 nm in diameter, and each has two small structures called kinetochores (Greek: *kineto* = movable; *chora* = space) (see Figure 10.3) that are attached to opposite sides of its centromere. A dense array of roughly parallel microtubules runs between the two poles of the spindle. This can be seen particularly clearly in Figure 10.9. These spindle microtubules have two ends, one of which is usually located within or near a pole. The other is either free within the spindle or is associated with a kinetochore. Microtubules emanate from each of the two poles, making the spindle a symmetrical structure that is formed by two opposing and overlapping arrays of microtubules. Each of these arrays is termed a half-spindle. In most vertebrates half-spindles contain 600-750 microtubules, of which 30%-40% end on kinetochores.

In addition to the microtubules in each half-spindle, other microtubules radiate outward from each pole (see Figure 10.61). These microtubules extend in all directions, forming a radial array called an aster that is centered on each pole. Like the spindle microtubules, all astral microtubules are oriented with one end at the pole while the other is located at a distant point within the cytoplasm. The asters play several roles during mitosis. In addition to positioning the spindle within the cell, which defines the plane of cytokinesis, they are also involved in separating the poles (centrosomes) during spindle formation and anaphase B.

The two kinetochores on each chromosome also play critical roles during mitosis. Their importance for chromosome motion was recognized very early because chromosome fragments lacking kinetochores cannot undergo directed

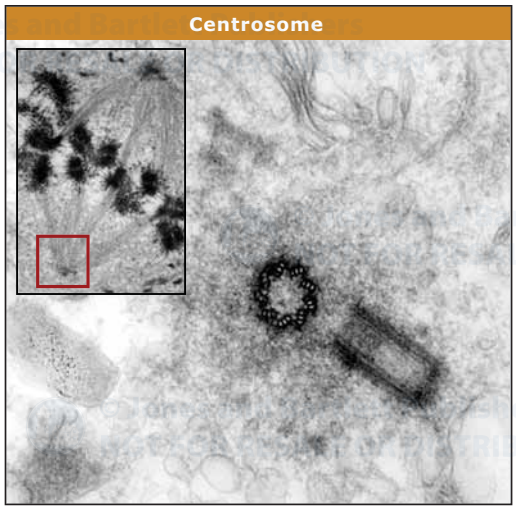

FIGURE 10.10 The large photograph shows an electron micrograph of a centrosome. The two centrioles are at right angles to one another so that one appears as a circle and the other as a rectangle. Around the first is a cloud of material that appears granular. (Compare the region immediately adjacent to the centriole with the more distant parts of the cytoplasm, which stain more lightly and where many membrane vesicles are visible.) Reprinted with permission from Rieder, C., and A. Khodjakov. 2003. *Science.* 300: 91-96. © 2003 AAAS. Photos courtesy of Conly Rieder, Wadsworth Center.

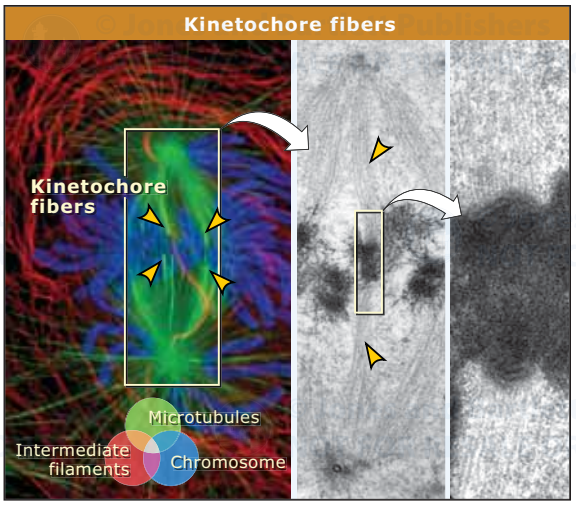

FIGURE 10.11 Kinetochore fibers on sister kinetochores seen by microtubule immunofluorescence (left) and electron microscopy (center and right). Photos © Conly Rieder, Wadsworth Center.

movements. Critical to their role is how they are positioned relative to one another. Because they are located on opposite sides of the centromere, they face different spindle poles, allowing a replicated chromosome to become attached to both poles. This positional relationship between the two kinetochores is essential for ensuring that the two chromatids become segregated into different nuclei. During spindle formation each kinetochore binds to the ends of multiple microtubules emanating from one of the poles, forming a bundle of microtubules called a kinetochore fiber that extends between it and the pole, as shown in FIGURE 10.11. Kinetochore fibers and kinetochores do not act simply as ropes and hooks that allow the chromatids to be pulled to the poles. Rather, through various interactions they play a vital active role not only in defining the direction the chromosome will move but also in generating the force for moving the chromosome.

The major questions that must be answered in order to understand mitosis at a molecular level are: How does the spindle form and how is its bipolarity ensured? How are the forces that move chromosomes produced and regulated? How is the fidelity of chromosome segregation ensured? How is the cytoplasm partitioned into two daughter cells after the chromosomes have been segregated?

10.4 Spindle formation and function depend on the dynamic behavior of microtubules and their associated motor proteins

Key concepts

- The spindle is a complex assembly of microtubules and microtubule-dependent motor proteins. The microtubules are highly organized with respect to their polarity.
- Spindle microtubules are very dynamic. Some exhibit dynamic instability, while others experience subunit flux.
- Interactions between microtubules and motors generate forces that are required to assemble the spindle.

The formation and proper function of the spindle depend on both the dynamic properties of its microtubules and the function of microtubule-dependent motor proteins. Although microtubules form the basic structural elements of the spindle, motor proteins are involved in organizing the microtubules into a spindle and in moving the chromosomes. Some motors play a direct role in assembling the spindle and linking its components into a coherent unit, while others are responsible for attaching the chromosomes to the spindle and generating forces for their motions. Even though the spindle has traditionally been regarded as a microtubule structure, it is more accurate to consider it as a collection of microtubules, motors, and other proteins.

Although motors play an essential role in generating forces within the spindle, the microtubules are far more than a static framework over which motors move. Throughout mitosis the microtubules are extremely dynamic, and this quality is essential both for assembling the spindle and for separating the chromosomes.

Within the spindle, microtubules are organized with respect to their polarity. As discussed in *7 Microtubules*, the two ends of a microtubule differ both chemically and structurally, imparting a structural "polarity" to the microtubule; a microtubule can be thought of as pointing in one direction or the other. The microtubules of each half-spindle, and those in its associated aster, are all arranged with the same polarity: their minus ends are near the pole while their plus ends are located at a distance from it, as shown in FIGURE 10.12. Where the two polarized arrays cross, their microtubules overlap, creating a region in the center of the spindle where adjacent microtubules are of opposite polarity. The uniform orientations of the microtubules in each opposing half-spindle are necessary for microtubule-dependent motors to participate in division. If the polarities of the microtubules within each half-spindle were random, different molecules of each type of motor would simply oppose one another, making any net movement chaotic, if not impossible.

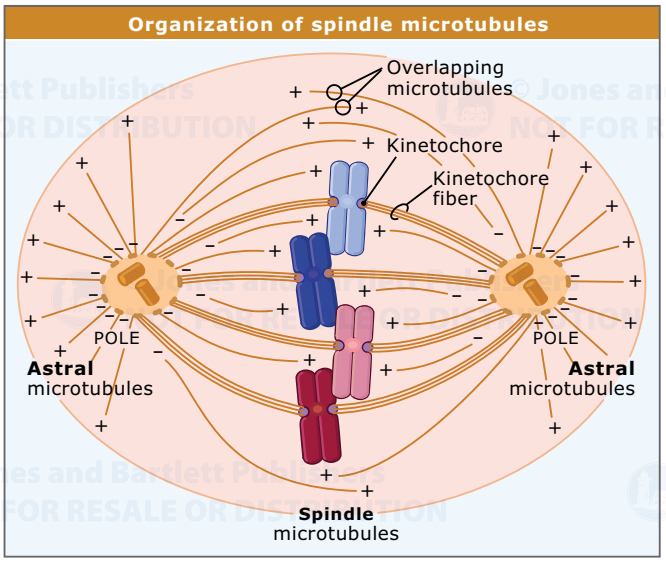

FIGURE 10.12 The microtubules within a spindle are organized with respect to their polarity. All have their minus ends near one of the two centrosomes and their plus ends at a distance from it. In the center of the spindle microtubules from the two centrosomes overlap, placing microtubules of opposite polarity adjacent to one another.

The dynamic properties of microtubules play an important role during all stages of mitosis. Work on cultured vertebrate cells and extracts made from the eggs of the frog *Xenopus laevis* reveals that the microtubules in each aster are undergoing dynamic instability and are shorter and much more dynamic than the microtubules in interphase cells. Some of this difference can be attributed to an increase during mitosis in the frequency of catastrophes, when microtubule plus ends switch from a growing or polymerizing state to a shrinking or depolymerizing state. It is also partly due to a decrease in the frequency of rescues, when depolymerizing or shrinking microtubules switch back to a polymerizing or growing state. *This increase in dynamics occurs as cells enter mitosis because microtubule-associated proteins that normally dampen catastrophe are inhibited, while others that promote microtubule growth are activated.* The balance between these two opposing activities is controlled by the master mitotic regulatory kinase, cyclin B/CDK1, which becomes active near the time of nuclear envelope breakdown (see *10.2 Mitosis is divided into stages*). As will be discussed next, the increase in microtubule dynamics that occurs as cells enter mitosis plays a major role in assembling the spindle.

As the spindle forms, a second type of microtubule dynamics arises. At this time, microtubules begin to exhibit a behavior known as subunit flux. In this curious form of behavior, tubulin subunits are incorporated at the plus end of a microtubule and then move through the microtubule to its minus end, where they are released. Flux occurs in all the microtubules of the spindle but is particularly prevalent in the microtubules within kinetochore fibers, as shown in FIGURE 10.13 and FIGURE 10.14. The origin of flux is unclear, but it may be due to the interaction of spindle microtubule plus and minus ends with other components (such

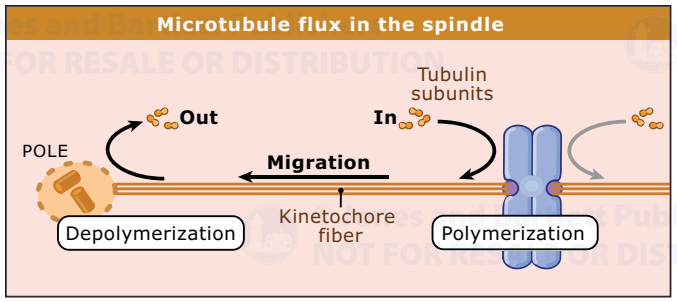

FIGURE 10.13 Tubulin subunits are continuously incorporated into microtubules at the kinetochores and move progressively toward the poles, where they are released. Tubulin subunits thus constantly flow from kinetochore to pole within the microtubules of a kinetochore fiber. During metaphase, the length of a kinetochore microtubule remains constant as long as subunit assembly at its plus end matches disassembly at its minus end. If subunit assembly at a kinetochore decreases with no change in disassembly at the pole, the kinetochore will move toward the pole. Flux is thus one possible means of moving a chromosome.

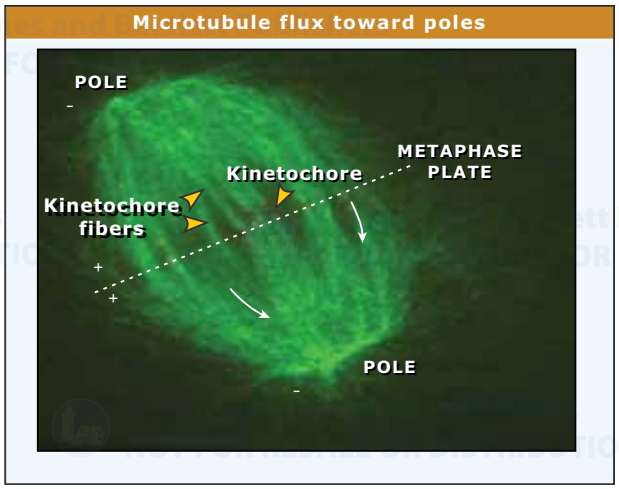

FIGURE 10.14 The first frame of a video (see CBIO: 10-0003) shows the mitotic spindle of a cell, with a small percentage of its tubulin fluorescently labeled (green). The kinetochores are in orange. The video shows the poleward flow of green dots along kinetochore fibers throughout the spindle. Photo courtesy of Paul Maddox, Ludwig Institute for Cancer Research.

as motor proteins) that are involved in organizing the spindle. Even as spindle microtubules undergo flux, astral microtubules continue to undergo dynamic instability. Although the purpose of flux is unknown, it likely plays a role in moving the chromosomes as well as in helping balance the forces within the spindle so that its two halves remain symmetrical.

Many different types of motor proteins interact within the spindle microtubule framework. Both the minus end-directed motor cytoplasmic dynein and motors of the kinesin superfamily (most of which move toward a microtubule's plus end) participate in mitosis. The spindle is sufficiently complex, and motors are so inherently involved in its formation and function, that *in higher organisms there are more than 15 kinesin family members that function only during the division process.*

Motor proteins are found throughout the spindle. This includes at the kinetochores, along the arms of the chromosomes, at the poles, and along the microtubules between the poles and the chromosomes. Many types of motors are found in only one location, but some are found in several. Cytoplasmic dynein, for example, is found at both the kinetochores and the poles, as well as in the cell's cortex, where it interacts with astral microtubules. The kinesin-like motor protein CENP-E, on the other hand, is concentrated in the kinetochore, while chromokinesins are found only on the arms of the chromosomes.

During mitosis, motor proteins perform several basic functions, as shown in **FIGURE 10.15**. Some, such as cytoplasmic dynein, bind to objects—including kinetochores and the plasma membrane—and move them in one direction along a microtubule (although in the case of the plasma membrane, it is the microtubule that actually moves). Others have multiple motor domains organized so that the motor can bind to two microtubules at once and crosslink them together. Depending on the structure of these motors, the adjacent microtubules may have the same or opposite polarity. If a motor binds to microtubules of opposite polarity, it will try to move (slide) them past one another until they no longer overlap. One example of this type of motor is the kinesin family member Eg5, which can bind to different microtubules at its two ends. Alternatively, if a motor is organized so that it binds to two microtubules with the same polarity, the result will be a collection of microtubules of the same polarity connected at one of their ends so that they form a radial array (like an aster). Other kinesin-related proteins do not move on microtubules but instead stimulate the disassembly of their plus ends. A good example is *m*itotic *c*entromere *a*ssociated *k*inesin (MCAK), which is found at the centromere of each chromosome. From motors with these basic properties, and the strategic positioning of different types of motors relative to one another, the spindle is constructed and the forces that move chromosomes are generated.

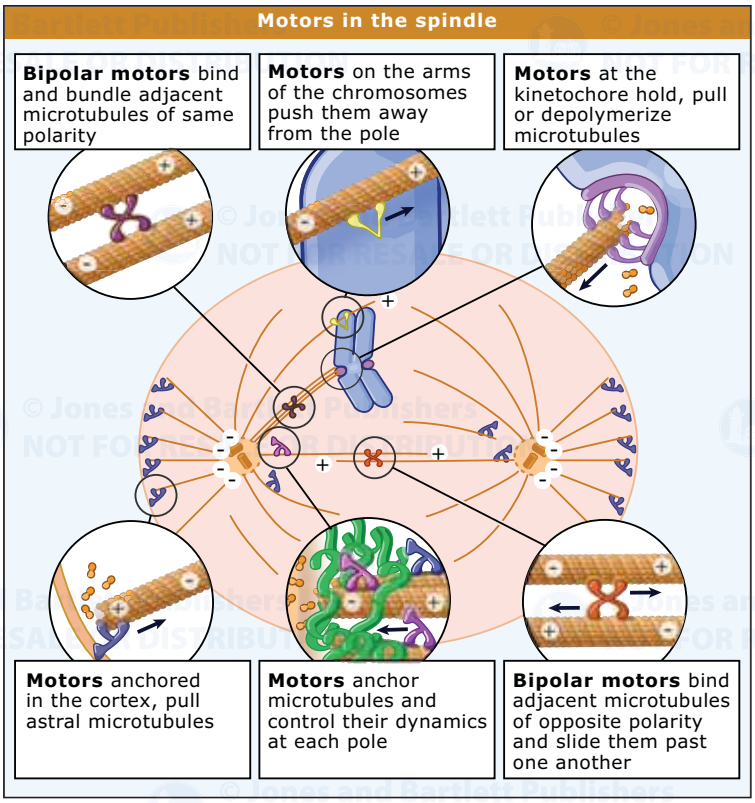

FIGURE 10.15 The spindle is packed with motor molecules that work on microtubules. Specific interactions between these motors and the microtubules form the spindle and are required for the movements it makes and the forces that it generates. Arrows indicate the direction in which a motor moves.

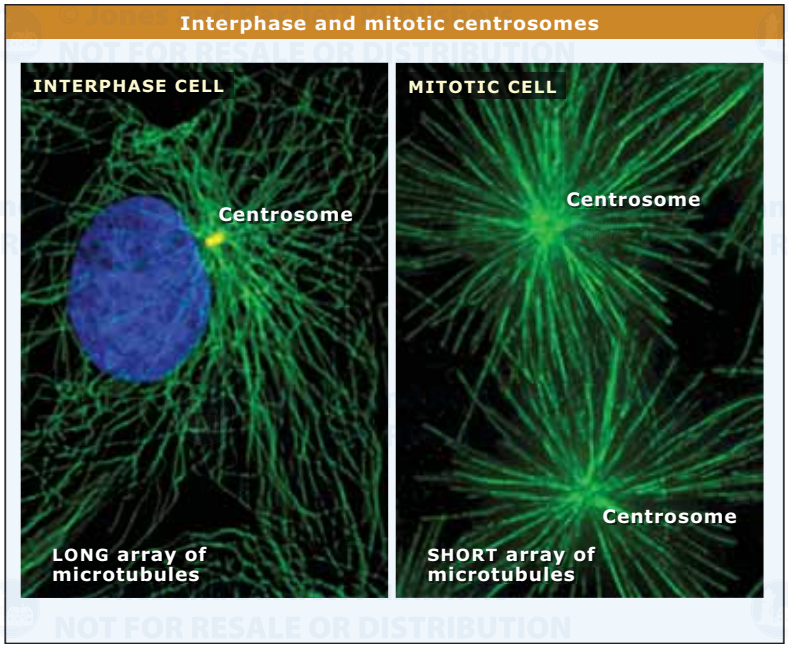

FIGURE 10.16 In interphase cells, the centrosome (the yellow dots near the nucleus) nucleates an extensive array of long microtubules that extend throughout the cytoplasm. In mitotic cells, the capacity of a centrosome to nucleate microtubules increases and each of the replicated centrosomes nucleates a dense radial (astral) array of short, straight microtubules. Microtubules are in green, DNA in blue. Photos © Conly Rieder, Wadsworth Center.

It is not always obvious how motors participate in spindle function. In some cases, for example, different motors are positioned such that they appear to oppose one another. Regardless of the details of the system, however, it is clear that *the formation and function of the spindle require multiple forces that must be balanced, and that those forces are generated by the activity of microtubule motors that work on a scaffold of dynamic spindle microtubules.*

10.5 Centrosomes are microtubule organizing centers

Key concepts

- Centrosomes define the poles of the spindle and play a role in spindle formation.
- Centrosomes nucleate microtubules and often remain bound to their minus ends afterward.

A number of changes occur in the cell near the time it becomes committed to mitosis. In animal cells, one of the most spectacular is that the interphase array of long cytoplasmic microtubules disappears and is replaced by two radial arrays of shorter microtubules, often referred to as asters. This conversion is shown in Figure 10.21. Each of these radial arrays surrounds a centrosome. The spindle in animal cells is formed from these two asters as the centrosomes separate. Since the two centrosomes will define the poles of the spindle, it is extremely important that two and only two are present during mitosis. As the two asters interact to begin the formation of the spindle, its structure is stabilized by the chromosomes, with a major role being played by their kinetochores.

When it is born, each cell contains a single centrosome that defined one of the spindle poles during the previous division. In nondividing cells, this minute organelle normally resides near the center of the cell, where it is closely associated with the nucleus. During interphase, the centrosome acts as a microtubule organizing center, generating and organizing an array of cytoplasmic microtubules that extends throughout the cell, as shown in FIGURE 10.16. These arrays are involved in organizing the cytoplasm and in moving material and organelles within the cell.

Centrosomes form microtubule arrays by acting as sites of microtubule nucleation. Within a centrosome, a microtubule begins to grow from a ring-shaped complex that contains a type of tubulin called γ-tubulin (see *7.7 Cells use mi-*

crotubule organizing centers to nucleate microtubule assembly). After a microtubule has been nucleated, its minus end usually remains anchored to the centrosome. The microtubule then elongates or shortens by the addition or removal of tubulin molecules primarily at its plus end, which is positioned at a distance from the centrosome. Microtubules remain anchored to the centrosome for variable periods and, in some cell types, are actively released by enzymes located within its structure. The anchoring mechanism involves several structural proteins, as well as minus end-directed motors, including cytoplasmic dynein and a member of the kinesin family (HSET). (For more on microtubule motors see *7.11 Introduction to microtubule-based motor proteins.*)

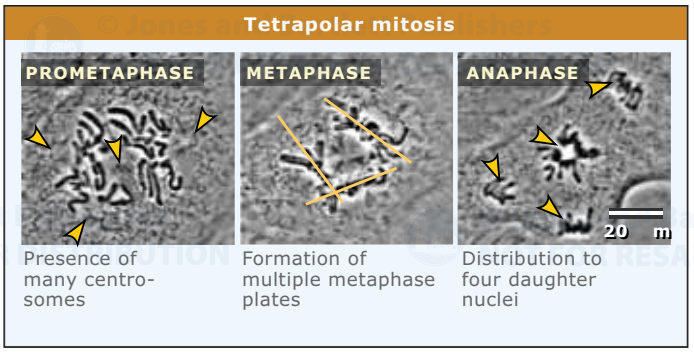

FIGURE 10.17 Mitosis in a rat kangaroo cell with four centrosomes (yellow arrowheads in the left panel). Three metaphase plates (yellow lines in the center panel) and four groups of chromosomes (arrowheads in the right panel) result. Cytokinesis will produce four aneuploid cells. Sluder, G., et al. *J. Cell Sci.* 1997. 110: 421-429. Used with permission from the Company of Biologists, Ltd. Photos courtesy of Conly Rieder, Wadsworth Center.

10.6 Centrosomes reproduce about the time the DNA is replicated

Key concepts

- Centrosomes are composed of two centrioles surrounded by the pericentriolar material.
- The formation of a new centrosome requires duplication of the centrioles.
- Centriole duplication is controlled by the cell cycle and is coordinated with DNA replication.
- Centrioles duplicate by the formation and growth of a new centriole immediately adjacent to each existing one.

During mitosis, every centrosome present in the cell has the potential to form a spindle pole. Spindles are normally bipolar only because cells usually enter mitosis with just two centrosomes. If a cell enters mitosis with more than two centrosomes, a spindle with too many poles will form and the cell stands a good chance of producing aneuploid progeny, as shown in **FIGURE 10.17**. To prevent this, mechanisms exist to ensure that the centrosome is replicated only once during the cell cycle. When these control mechanisms break down, too many centrosomes are formed, which may lead to genetic defects that result in cancer cells and tumors. In order to understand how the bipolar nature of a normal spindle is ensured, it is necessary to examine the structure of the centrosome and how the cell controls its replication.

Under the light microscope the centrosome appears in most living cells as one or two dots. To reveal more detail and fully appreciate the structural complexity of this organelle, the electron microscope (EM) is used. With this tool, the core of the centrosome is seen to contain a pair of structures called centrioles, as shown in **FIGURE 10.18**. Each centriole consists of a pinwheel of nine triplet microtubule blades distributed evenly around the perimeter of a cylinder ~0.3 µm in diameter. As early as 1888, Boveri and others concluded that the centriole, which was just barely visible with their light microscopes, was a permanent and independent organelle formed only by the division of a preexisting centriole. Indeed, with few exceptions, new centrioles are only formed in association with the wall of an existing centriole. In some cells, this close physical relationship between the two centrioles persists throughout the cell cycle. However, in many cells it is lost during interphase, and the two centrioles wander independently throughout the cell.

Each centriole is associated with a diffuse cloud of material that appears in the EM as an opaque substance. It is clearly visible around the centriole seen "end-on" in Figure 10.10. This pericentriolar material consists of a large number of proteins attached to a scaffold. As a rule, the older (mother) centriole contains more of this material than its daughter, at least until the next round of centriole replication is completed. Among the proteins included in the pericentriolar material are several types of microtubule-dependent molecular motors and the γ-tubulin rings used in microtubule nucle-

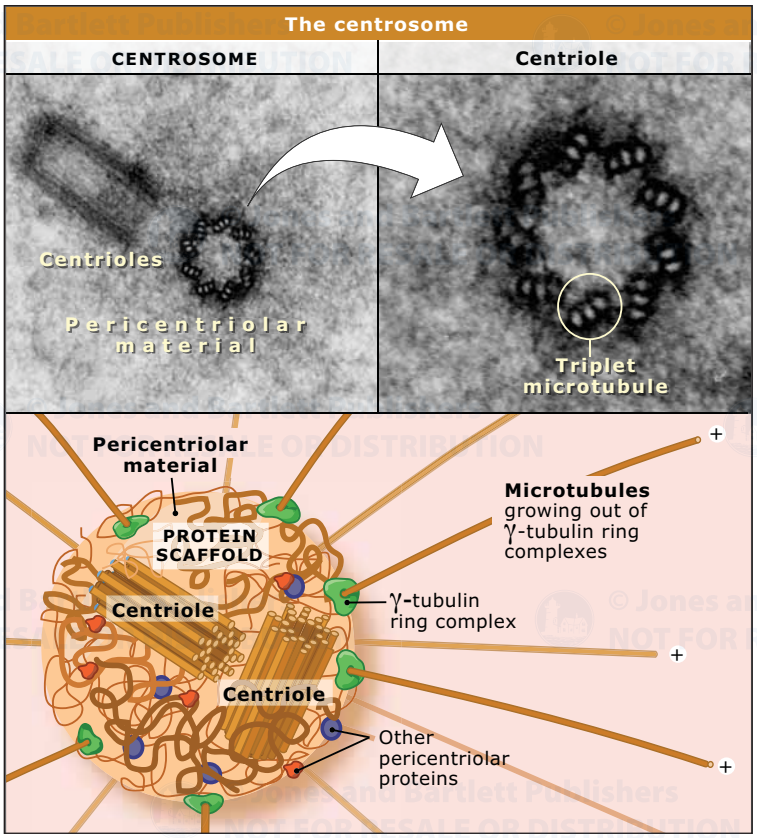

FIGURE 10.18 The centrosome in a mitotic cell (upper left) consists of two centrioles—a mother (cut in cross section in this electron micrograph) and a daughter (cut lengthwise)—surrounded by a cloud of amorphous pericentriolar material. Each centriole (upper right) is composed of triplet microtubules arranged as the wall of a cylinder. The drawing shows how the centrioles are positioned at right angles to one another, with many different proteins collected around them to form the pericentriolar material. Photos © Conly Rieder, Wadsworth Center.

ation (see Figure 10.18). The centrioles themselves also contain a number of specific structural and enzymatic proteins, some of which are also present in the pericentriolar material.

During the interphase portion of the cell cycle, the centrosome serves several concurrent functions. In most cells, during G1, the mother centriole initiates the formation of a long, thin, membrane-enclosed structure called a primary cilium, which projects from the surface of the cell, as shown in **FIGURE 10.19**. Although often ignored, primary cilia are so common that it is easier to list the cells that lack them than those that possess them. In some epithelia these structures can protrude more than 20 μm from the dorsal cell surface. Since they are not found in many transformed cells, primary cilia are not essential for cell survival, a fact that initially led scientists to speculate that they are simply vestigial appendages like the appendix. However, the outer segments of the rod and cone cells of the eye—the highly specialized structures where photons are absorbed—are formed from derivatives of primary cilia, and evidence is rapidly accumulating that primary cilia are also required for proper development and tissue function.

In animal cells, the number of centriole pairs defines the number of centrosomes. *Cells, therefore, control the number of centrosomes they contain by regulating the replication of centrioles.* Research is just beginning to uncover the mechanisms that control the precise doubling of centrioles and how this reproduction is coordinated with the nuclear activity of the cell cycle (e.g., DNA replication). It is now evident that the timing of centriole replication is governed by changes in the cytoplasm, indicating that a soluble factor regulates their duplication. In addition, conditions that allow centrioles to replicate are found only during the S phase of the cell cycle, when the cell's DNA is also being replicated. The primary regulator of centrosome replication appears to be the CDK2 kinase and its cyclin A and E activators. These regulators become active near the beginning of S phase and are also responsible for driving the cell into DNA synthesis (for details see *11 Cell cycle regulation*). The fact that the same regulator initiates the replication of both DNA and centrioles ensures that these two activities are coordinated, so that a cell enters mitosis having replicated both its centrosome and its chromosomes. Although it is clear how the duplication of centrioles is initiated, it is not yet clear how their replication is limited to the formation of a single new centriole from each preexisting one.

Once started, centriole duplication proceeds by the gradual formation of a new centriole adjacent to each of the two centrioles with which the cell entered the S phase. Of those, one, termed the daughter, is younger because it was formed in the previous cell cycle. The other, termed the mother, was formed in an earlier cell cycle and carries the primary cilium (see Figure 10.19). The first sign of centriole replication is the appearance of two short procentrioles, each of which extends at a right angle from the wall of one of the existing centrioles, as shown in **FIGURE 10.20**. This process does not depend on a physical relationship between the two original centrioles because it can occur even when they are separated. Once procentrioles

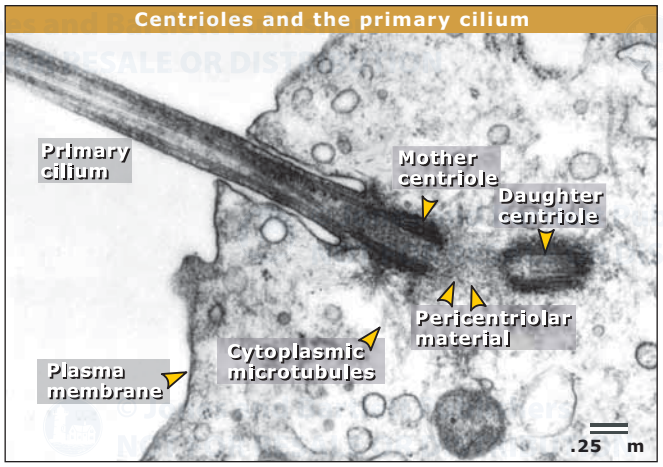

FIGURE 10.19 An electron micrograph of the base of a primary cilium. The structure projects from the mother centriole, which is located immediately beneath the plasma membrane. The mother and daughter centrioles are connected by the pericentriolar material, visible as dense, granular material extending between the two. Only a small part of the entire cell and the length of the primary cilium are shown. Photo © Conly Rieder, Wadsworth Center.

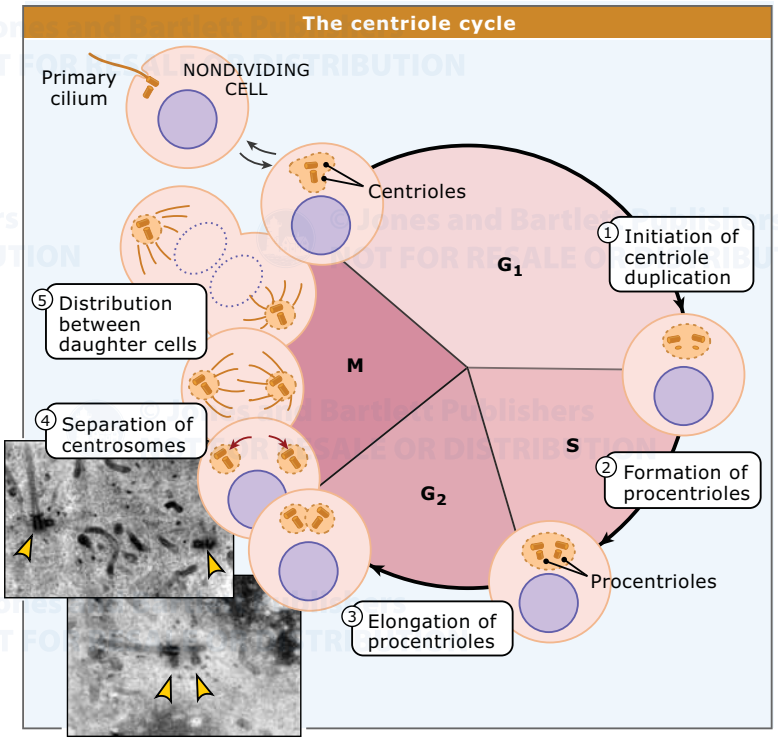

FIGURE 10.20 The centriole cycle in a mammalian cell. The two centrioles in the mother cell's centrosome are duplicated and the two centrosomes that result are then separated into the daughter cells. The insets show electron micrographs of replicated centrosomes before and after they separate early in mitosis. Reprinted from *J. Ultrastruct. Res.*, vol. 68, Rieder, C., et al., *The resorption of primary cilia...*, pp. 173-185. Copyright 1979, with permission from Elsevier. Photos courtesy of Conly Rieder, Wadsworth Center..

have formed, they slowly elongate until they reach the length of a mature centriole near the time of mitosis. Although the mother and daughter centrioles form and grow procentrioles identically, most of the pericentriolar material remains associated with the more mature mother centriole. The daughter centriole ultimately gathers new pericentriolar material during the duplication process, in part from the microtubule array that it organizes. By late interphase the cell contains two centrosomes, each containing a pair of closely associated centrioles and their pericentriolar material. In some cells these two centrosomes remain physically tethered together and function as a single unit until the cell enters mitosis. In other cells this connection is broken, and the two centrosomes move apart before there is any visual evidence that the cell is entering mitosis. The timing of when the two centrosomes separate with respect to nuclear envelope breakdown is highly variable, even in genetically identical cells that are adjacent to one another.

10.7 Spindles begin to form as separating asters interact

Key concepts

- As mitosis begins, changes in both the centrosomes and the cytoplasm cause a radial array of short, highly dynamic microtubules to form around each centrosome.
- Interactions between the asters formed by the two centrosomes initiate the formation of the mitotic spindle.
- Separation of the centrosomes depends on microtubule-dependent motor proteins.
- The pathway of spindle formation depends on whether the centrosomes separate before or after the nuclear envelope breaks down.

As cells progress from interphase into mitosis, the distribution of microtubules goes through a rapid and striking change. The array of long cytoplasmic microtubules typical of interphase cells disassembles, and each of the two centrosomes nucleates a dense radial array of shorter

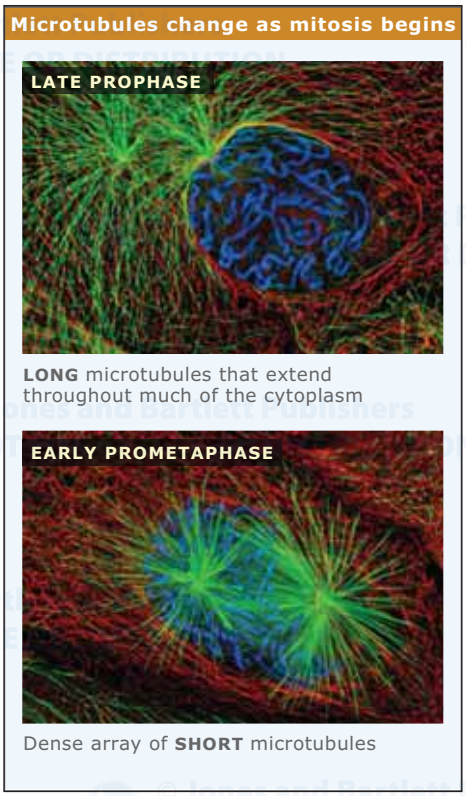

FIGURE 10.21 The conversion from one array to the other takes only a few minutes. Microtubules are in green, chromosomes in blue, and intermediate filaments in red. Photos © Conly Rieder and Alexey Khodjakov, Wadsworth Center.

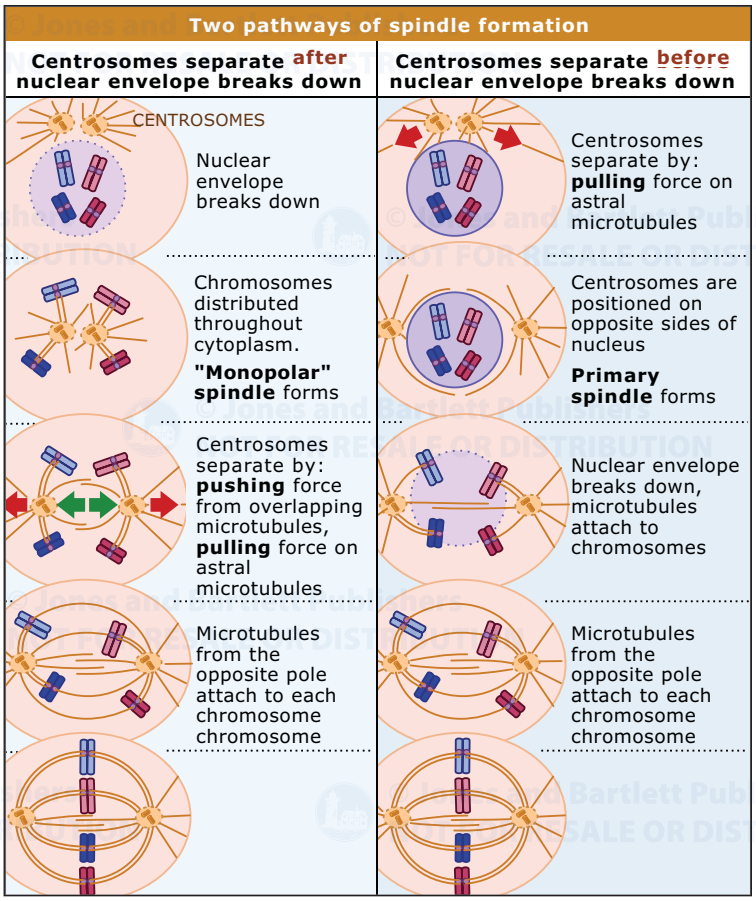

FIGURE 10.23 The two pathways differ in when the centrosomes separate relative to nuclear envelope breakdown. The ability of a spindle to form regardless of when the centrosomes separate emphasizes how remarkably flexible the process of spindle formation is.

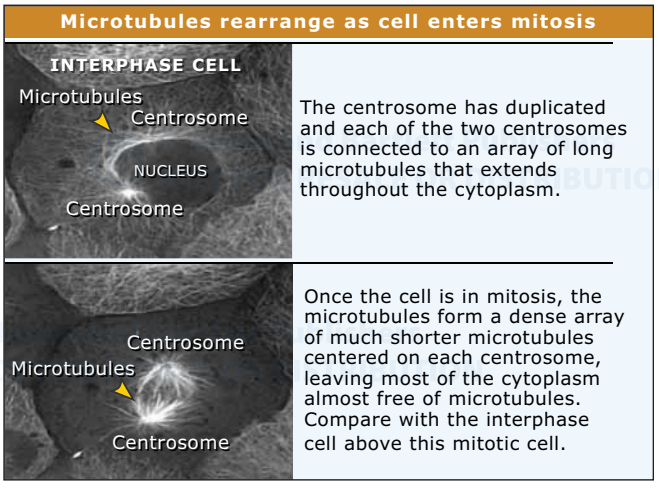

FIGURE 10.22 The photos are from a movie that shows the conversion of a microtubule array into a mitotic array. Photos courtesy of Patricia Wadsworth, University of Massachusetts, Amherst.

microtubules, as shown in FIGURE 10.21 (see also Figure 10.16). As illustrated in FIGURE 10.22, these two asters will ultimately contribute the microtubules for spindle formation. The changes in number and distribution of microtubules at the onset of mitosis are mediated both by changes within the centrosomes and by others that occur throughout the cell.

Near the time when the cell becomes committed to mitosis, the two centrosomes change so that they are capable of nucleating many more microtubules than during interphase. As this happens, proteins associated with the centrosome become heavily phosphorylated, the centrosome's γ-tubulin content increases, and the pericentriolar material surrounding the centrioles expands. It is not clear how this "maturation" process occurs; however, it probably involves specific kinases activated as a cell progresses from G2 into M phase, including the master mitotic regulatory kinase (for details see *11 Cell cycle regulation*).

At about the same time, due to changing conditions in the cytoplasm, microtubules be-

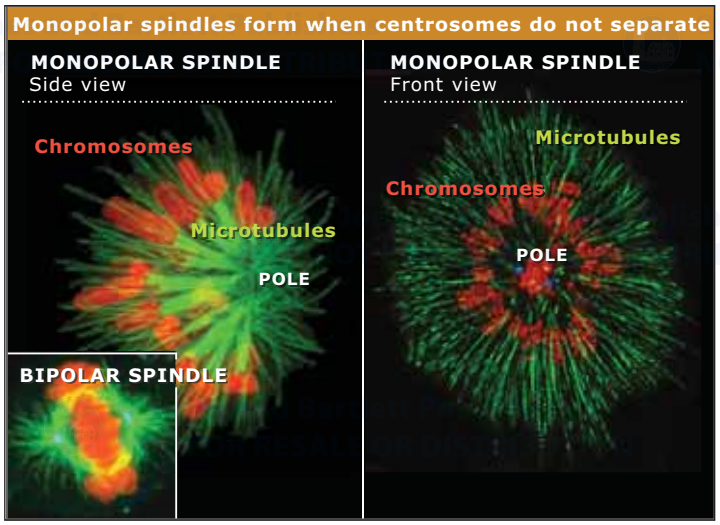

FIGURE 10.24 On the left is a side view of the monopolar spindle formed in a rat kangaroo cell when the centrosomes were prevented from separating. The chromosomes (orange) attach to the single polar region. Note the thick kinetochore fibers. A normal bipolar spindle is shown in the inset for comparison. An end-on view of a similar monopolar spindle in a human cell is shown on the right. The centrosomes are in blue in the center. Photo © Conly Rieder, Wadsworth Center (left); photo courtesy of Alexei Mikhailov, Wadsworth Center (right); and Canman, J.C., et al. *Nature*. August, 2003. 424; 1074-8. Photo courtesy of J.C. Canman and E.D. Salmon, University of North Carolina at Chapel Hill (inset).

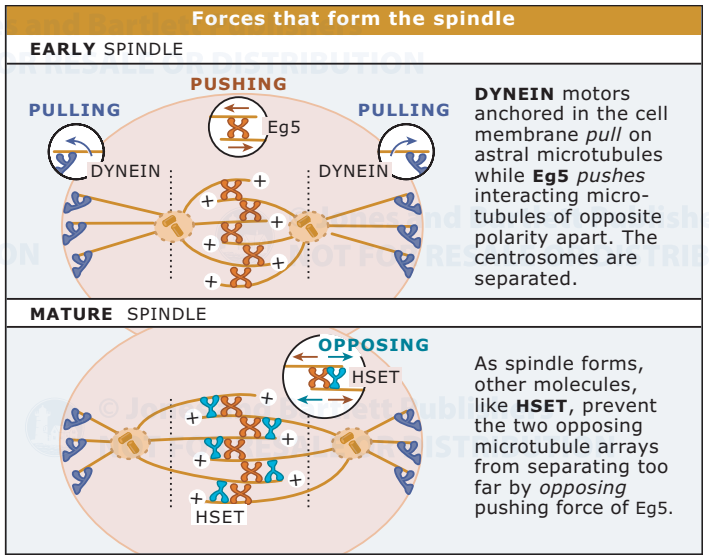

FIGURE 10.25 The process begins as soon as the nuclear envelope breaks down. Initially the two centrosomes are immediately adjacent to one another. Both Eg5 and HSET are members of the kinesin family of microtubule-dependent motors, but Eg5 moves toward the plus (+) end of a microtubule and HSET toward the minus (–) end. The spindle's length is determined by a balance among the forces of the three motors.

come less stable, leading to the replacement of the long interphase array by two shorter astral arrays growing from the centrosomes, as discussed earlier. The result is that by the start of prometaphase, the total length of microtubules in the cell has decreased, and the rate at which new microtubules are formed and old microtubules disassemble (i.e., turn over) has increased. This means that as the nuclear envelope begins to break down, the region surrounding the nucleus is constantly being probed by a large number of very dynamic microtubules growing from each aster. As we will see, this dynamic behavior facilitates connecting the asters with the chromosomes.

As the two centrosomes separate, interactions between the astral microtubule arrays growing from them begin to form the spindle. Spindle formation is a remarkably flexible process that can occur by either of two pathways, depending on whether the centrosomes separate before or after the nuclear envelope breaks down, as shown in FIGURE 10.23. Both pathways involve the interaction of microtubules with motor proteins.

In cases where the nuclear envelope dissolves before the centrosomes have begun to separate, the liberated chromosomes are distributed throughout the cytoplasm and are exposed to only a single large aster. As FIGURE 10.24 shows, a "monopolar" or half-spindle is formed; it lasts until the two centrosomes finally separate and convert it into a bipolar spindle. The separation of centrosomes after nuclear envelope breakdown involves two forces: one that pushes them apart, caused by the activity of the kinesin-like protein Eg5 as it interacts with adjacent microtubules of opposite polarity between the two centrosomes, and a second that pulls, caused by the activity of cytoplasmic dynein anchored at the periphery of the cell (i.e., in the cortex), as shown in FIGURE 10.25 (for more on kinesin and dynein see *7.11 Introduction to microtubule-based motor proteins*). If unopposed, these pushing and pulling forces move the centrosomes apart until the two astral arrays no longer overlap. However, separation of the centrosomes is restricted by other motors that bind to the overlapping microtubule arrays, as well as by the formation of kinetochore fibers on sis-

ter kinetochores (which tether the two centrosomes to one another, as we will see in *10.8 Spindles require chromosomes for stabilization and can "self-organize" without centrosomes*).

The spindle forms via a different pathway if the two asters are already separated by the time the nuclear envelope breaks down, as shown in Figure 10.23. Under this condition, the separation of the asters does not involve Eg5, which is not available because it is located in the nucleus. Instead, cytoplasmic dynein interacts with the microtubules growing from each centrosome and, once the linkage between the two centrosomes is severed, is able to pull them apart. In this case, dynein is located both in the cortex and on the surface of the nuclear envelope. Actin filaments define the directions in which the two centrosomes move through interactions, with myosin located either in the centrosomes or along the microtubules.

When the two asters separate before nuclear envelope breakdown, a primary spindle often forms in the region where their opposing microtubule arrays overlap. However, until the nuclear envelope breaks down, this structure is not stable, and the microtubule arrays of the separating centrosomes can be pulled far enough apart so that they no longer overlap. The reason for this is that the stability of the spindle requires proteins that are sequestered in the nucleus during interphase and are only released into the cytoplasm after nuclear envelope breakdown. As a result, in many late prophase cells the two centrosomes and their associated asters are positioned on opposite sides of the nucleus, with no interaction between them. In these cells, the spindle only forms after the kinetochores become accessible to microtubules and can relink the two asters.

10.8 Spindles require chromosomes for stabilization but can "self-organize" without centrosomes

Key concepts

- Adjacent asters will separate completely and fail to form a spindle in the absence of chromosomes.
- Chromosomes stabilize both the basic geometry of the spindle and the microtubules in it by binding astral microtubules at their kinetochores.
- Spindles can form in the absence of centrosomes, although they form more slowly and lack astral microtubules.
- A centrosomal spindle formation involves nucleation of microtubules by the chromosomes and the functions of several different types of microtubule-dependent motor proteins.

As it forms, the spindle is stabilized by the chromosomes and their kinetochores and by molecular motors that bind to the microtubules and attract additional components into the spindle.

Particularly important for establishing the basic shape of a spindle are the motor proteins that connect neighboring microtubules of opposite polarities. Such microtubules are present where two asters overlap, and crosslinking them creates a spindle-shaped structure similar in length to a normal spindle even if there are no chromosomes nearby. *However, spindles that lack chromosomes are not stable and progressively lose their microtubules.*

How does the association of chromosomes with the nascent spindle prevent the loss of microtubules? The answer is not fully understood, but it appears to involve several different overlapping mechanisms. Each of the kinetochores on a chromosome recruits astral microtubules into a specialized bundle called a kinetochore fiber that connects the kinetochore to a pole (discussed in *10.9 The centromere is a specialized region on the chromosome that contains the kinetochores*). Inclusion in a kinetochore fiber increases the stability of those microtubules relative to other microtubules in the spindle. This happens to a significant fraction of the microtubules in each aster; by metaphase, ~30%-40% of the 1200-1500 microtubules in a typical spindle are stabilized by attachment to a kinetochore. Because there are two kinetochores on each chromosome, the formation of kinetochore fibers tethers the two spindle poles together, causing the opposing astral arrays of microtubules to interact even more.

While kinetochore fibers are being formed, each aster is also recruiting and concentrating a wide variety of proteins that help stabilize its microtubules. Some of these are structural proteins that are organized into a loose spindle matrix that surrounds the microtubules (see Figure 10.39). For example, when the *nu*clear *m*itotic *a*pparatus (NuMA) protein is released from the nucleus and phosphorylated by CDK1, it becomes concentrated within the spindle. By binding various motor molecules in the spindle, NuMA anchors and stabilizes microtubules. The stabilizing influence of matrix components is significant, but is not nearly as great as that of kinetochores. In a mature spindle, the life-

time of a microtubule associated with a kinetochore is 10 times longer than for other spindle microtubules.

Surprisingly, bipolar spindles can form in the absence of centrosomes. This occurs through a remarkable process of self-organization in which randomly nucleated microtubules are assembled into a bipolar structure by the chromosomes and microtubule-dependent motor proteins. This "acentrosomal" route for spindle assembly is used by all higher plants and is also found during meiosis and the early developmental stages of some animals. One possibility is that it is an evolutionary ancestor of centrosome-mediated spindle formation, and that it is normally masked by the presence of centrosomes. This idea is supported by the fact that even animal tissue cells, which normally contain centrosomes, can construct a bipolar spindle in their absence. This is an excellent example of how cells have evolved multiple mechanisms by which to accomplish the same task.

During acentrosomal spindle assembly, short microtubules form near each chromosome via a pathway that involves proteins on the chromosome surface. These microtubules are initially randomly oriented but are then organized into parallel arrays by the action of microtubule-dependent motor proteins, as shown in FIGURE 10.26. A central role is played by motors that bind two oppositely oriented microtubules at once and simultaneously move toward the plus end of each. These proteins first align the microtubules around a chromosome simply by binding along their length and crosslinking them. By then moving toward the plus end of each microtubule, these motors sort them into two groups of parallel, polarized microtubules that meet at their plus ends. The motor proteins may remain bound there, helping maintain the structure. Motor proteins on the arms of the chromosomes—called chromokinesins—also participate. Part of their role may be to tether the microtubules in the vicinity of the chromosomes so that they remain at the center of the spindle as it forms. Once sorted, the microtubules within each of the two arrays become bunched together at their minus ends by minus end-directed motors (like cytoplasmic dynein and HSET), thus giving the entire array the shape of a spindle. This self-assembly process takes place independently around each chromosome. The multiple spindles thus formed then fuse into a single large spindle with broad poles. Structural proteins and other matrix components—like NuMA—that are transported toward the minus ends of microtubules (i.e., toward the forming pole) glue the ensemble together and stabilize the polar regions.

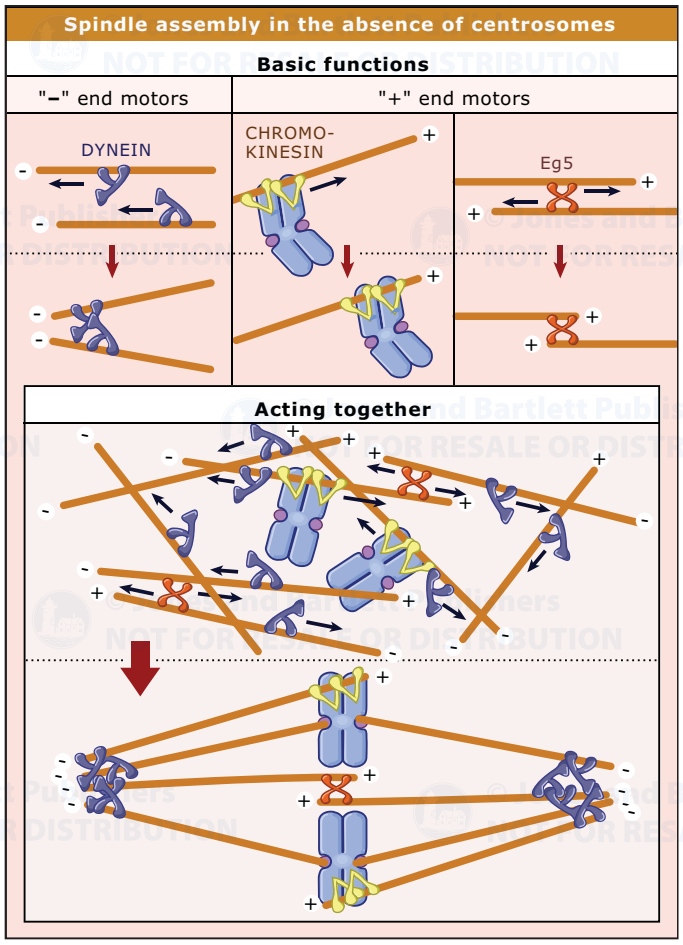

FIGURE 10.26 Microtubules are nucleated with random orientations around the chromosomes. Once microtubules have formed, three types of motors work together to organize them into a bipolar array around the chromosomes.

If centrosomes are not needed to form a bipolar spindle, why are they found at the spindle poles during mitosis in most animal cells? One reason is that centrosomes provide a kinetic advantage and allow the spindle to form more quickly. This is important because, during the development of an organism, spindles must often form synchronously and very rapidly. Another reason is that the poles of spindles formed by centrosomes contain asters, which are not present in the polar regions of spindles formed by the acentrosomal route. These asters define the position of the cleavage furrow during cytokinesis by positioning the spindle within the cell. This aspect of aster function is critical for proper development. Lastly, in addition to its role as a microtubule organizing center,the centrosome plays other roles in the cell (e.g.,

in primary cilia formation and cell cycle progression). Associating a centrosome with each spindle pole is a convenient and reliable mechanism to ensure that each new cell will inherit a copy of this important organelle.

10.9 The centromere is a specialized region on the chromosome that contains the kinetochores

Key concepts

- Proper attachment of the chromosomes to the spindle is required for their accurate segregation.
- Attachment occurs at the kinetochores, where the chromosomes interact with the spindle's microtubules.
- The centromere is the site where the two kinetochores on each chromosome form.
- Each chromosome has a single centromeric region.
- Centromeres lack genes and are composed of highly specialized, repetitive DNA sequences that bind a unique set of proteins.

One the most critical events of mitosis is getting the chromosomes properly attached to the spindle. Improper attachments lead to errors in chromosome segregation, with potentially disastrous consequences for the organism. Much of the reliability of the attachment process results from the properties of the kinetochores, small structures on each chromosome specialized for interacting with microtubules.

Attachment occurs at the centromere, a region on each chromosome that is specialized for the purpose. The centromere is clearly visible with the light microscope as a constricted region on the condensed chromosome (see Figure 10.3). The constriction gives mitotic chromosomes their characteristic shape. Depending on the chromosome, the centromere may be located near the middle of a chromosome (metacentric), near its end (acrocentric), or somewhere in between (submetacentric). Its position within a chromosome does not change.

The centromere region differs chemically from the rest of the chromosome. It contains few if any genes, and is composed almost entirely of a large number of highly repetitive DNA sequences called α satellite repeats, or satellite DNA. A unique group of proteins called *centromere proteins*, or CENPs, associate with these sequences. The CENPs include CENP-A, which is a modified form of histone H3; CENP-B and CENP-G, which are involved in packaging the satellite DNA; and CENP-C, the function of which is unknown. Most of the CENPs play structural roles, binding to the repeat sequences and organizing them into a highly compacted form of chromatin called heterochromatin that is found nowhere else in the chromosome.

In addition to the CENPs the centromere region also contains another class of proteins known as chromosomal passengers. Passenger proteins are unusual in that they change their location during the course of mitosis. Unlike the CENPs, which remain at the centromere throughout mitosis, the passenger proteins are present at the centromere during prophase and metaphase but relocate onto microtubules between the two groups of separating chromosomes as anaphase begins. All of the passenger proteins that have so far been identified appear to form a single complex within the centromere that includes the aurora B kinase, an enzyme whose inactivation has a dramatic effect on spindle organization. This complex is important for correcting errors in kinetochore attachment early in mitosis, a role it must play while located at the centromere. Much later, after the complex has moved onto the microtubules in the spindle midzone, it is thought to participate in cytokinesis.

In addition to the CENPs and passenger proteins, the centromere region also contains a kinesin-related protein. Unlike most members of the kinesin family, which move along the sides of microtubules, this protein stimulates microtubule plus ends to disassemble (for more on kinesin see *7.12 How motor proteins work*). Recent evidence suggests that this motor protein, along with its Aurora kinase activator, is involved in correcting errors in kinetochore attachment.

Perhaps the most important role of the centromere is to organize the kinetochores on the chromosome. Kinetochores are highly defined structures and the surface of each centromere is specialized for their assembly. They are extremely small relative to the size of the entire chromosome and their shape and internal structure can only be seen when they are viewed with an electron microscope (as in Figure 10.9). Each centromere forms two kinetochores, positioned precisely on opposite sides of the chromosome. The kinetochores are the site where the chromosomes are actually attached to the spindle, and their precise positioning relative to one another on each chromosome is fundamentally important to the success of mitosis.

10.10 Kinetochores form at the onset of prometaphase and contain microtubule motor proteins

Key concepts

- Kinetochores change structure as mitosis begins, forming a flat plate or mat on the surface of the centromere.
- Unattached kinetochores have fibers extending out from them (the corona) that contain many proteins that interact with microtubules.
- The corona helps kinetochores capture microtubules.

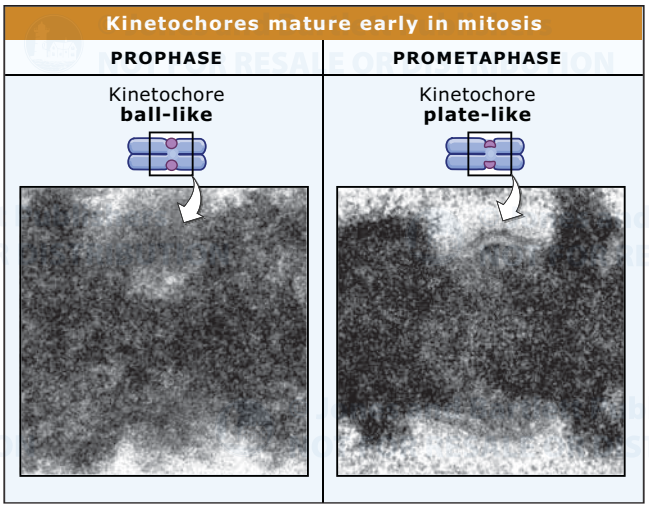

FIGURE 10.27 Kinetochores in a rat kangaroo cell during prophase (left) and prometaphase (right). The prometaphase kinetochore has no microtubules attached to it because microtubule assembly was inhibited in the cell before the picture was taken. The kinetochore changes from a ball of material in prophase to a plate-like structure in prometaphase. Photos © Conly Rieder, Wadsworth Center.

Associated with each centromere are two "sister" kinetochores arranged back-to-back so that they are on opposite sides of the centromere and face in opposite directions. This back-to-back positioning of sister kinetochores helps to ensure both that each attaches to only one pole, and that they attach to different poles. A chromosome's sister chromatids will only move to opposite poles of the spindle if the chromosome has acquired this proper bipolar attachment. The structure and composition of kinetochores is complex and changes both over the course of the cell cycle and during the various stages of mitosis.

The composition of the kinetochore remained a mystery until the early 1980s. At that time it was discovered that some patients with an autoimmune disease called CREST syndrome (a variant of systemic sclerosis) have antibodies to kinetochore proteins in their blood. Immunofluorescence studies with these antibodies showed pairs of adjacent dots in the nuclei of interphase cells that had replicated their chromosomes, with the number of pairs of dots in each nucleus equal to the number of chromosomes. These antibodies identify precursor structures present in interphase cells that will mature into mitotic kinetochores. These "prekinetochores" contain several of the CENPs and appear in the electron microscope as spheres of tightly packed fibrillar material embedded in the heterochromatin of the centromere. As the cell enters mitosis and the chromosomes begin to condense, additional components associate with the prekinetochores.

Still more components are added to the prekinetochore when the nuclear envelope breaks down, and it undergoes a physical change as a result. *The spherical mass of fibers characteristic of the prophase kinetochore is replaced by a very thin (50-75 nm thick) circular or sometimes rectangular fibrous plate or "mat,"* as shown in FIGURE 10.27, on the surface of the centromere. The diameter of this new form of the kinetochore is usually about 0.2 to 0.5 μm, although it varies considerably, even on different chromosomes within the same cell. (For comparison, a microtubule is about 0.025 μm in diameter and a mitotic chromosome can be up to about 40 μm from end to end.) Several proteins that are important for proper assembly of the plate, including CENP-A and CENP-C, are found on the surface of the centromere to which the mat is attached.

When kinetochores are not attached to microtubules, a dense network of thin fibers extends outward from the cytoplasmic surface of the kinetochore mat. This network is known collectively as the corona material. Several proteins important to kinetochore function are found in the corona. These include cytoplasmic dynein (a minus-end microtubule motor), CENP-E (a member of the kinesin family and a plus-end microtubule motor), and several additional proteins that facilitate the attachment of microtubules to kinetochores, including at least one +TIP. In addition, the corona also contains several components of a cell cycle checkpoint that monitors the assembly of the spindle. Most of the proteins found in the corona are dynamically associated with it, constantly dissociating and later rebinding. This continuous turnover makes the corona a steady state struc-

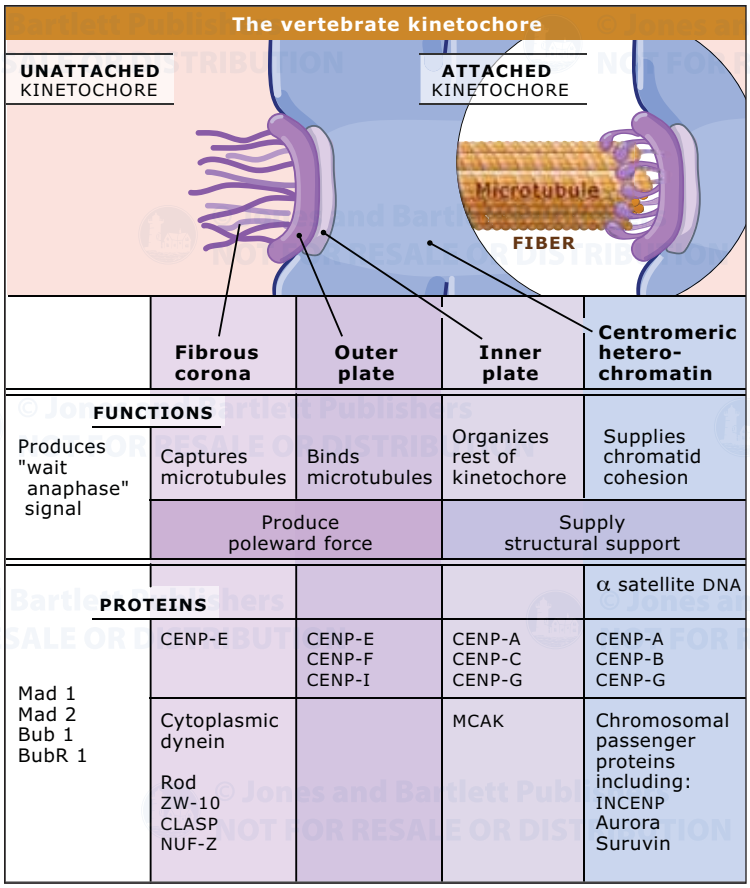

FIGURE 10.28 The functions of the different parts of the vertebrate kinetochore. The number of different proteins found in each part indicates how complex the structure is.

ture, its overall form and composition remaining the same but its individual components continuously changing.

The function of the corona is, in part, to help the kinetochore capture microtubules. The presence of the corona in early mitosis greatly increases the surface area of the kinetochore during the period when the spindle is forming and the chromosomes must become attached to it. The concentration within the corona of motors and other proteins that bind microtubules accelerates the attachment process by creating a large surface around each kinetochore capable of catching and trapping microtubules much as flypaper traps flies.

As a kinetochore acquires microtubules and becomes attached to the spindle, many of the components of the corona begin to disappear and/or become redistributed. At the same time, the amount of kinetochore-associated molecular motor proteins also decreases. These proteins reappear, however, if the microtubules associated with the kinetochore are removed by disassembling them with drugs.

The composition of the various parts of the kinetochore and the role each plays are shown in **FIGURE 10.28**. The number of different proteins in each of the parts emphasizes how complex a structure the kinetochore is. Note that throughout it there are proteins that interact with microtubules.

10.11 Kinetochores capture and stabilize their associated microtubules

Key concepts

- Kinetochores and microtubules become connected by a search-and-capture mechanism made possible by the dynamic instability of the microtubules. The search-and-capture mechanism gives spindle assembly great flexibility.
- Capturing a microtubule causes a kinetochore to move poleward. This expedites the capture of additional microtubules and starts the formation of a kinetochore fiber. .
- One sister kinetochore usually captures microtubules and develops a kinetochore fiber before the other does
- The ability of kinetochores to stabilize associated microtubules is essential for the formation of a kinetochore fiber.
- Kinetochores under tension are much more effective at stabilizing microtubules than kinetochores that are not under tension.

The attachment of a chromosome to the spindle requires that each of its kinetochores bind microtubules from one of the two centrosomes. How do astral microtubules and kinetochores actually find one another to allow attachment to occur? This is a very demanding spatial problem for the cell to solve reliably. Chromosomes are very large and diffuse very slowly, so the kinetochore cannot move to aid the process. It is, therefore, a stationary target that must be found by microtubules. Considered on the scale of the cell kinetochores are very small, and all 92 of them (in the case of human cells) must be found and associated with microtubules if the chromosomes are to be segregated properly. The problem is compounded by the fact that, as mitosis begins, the kinetochores are in unpredictable locations. After nuclear envelope breakdown the chromosomes are distributed throughout the cytoplasm, with their positions and orientations different from cell to cell and division to division. A spindle must be formed correctly regardless of how the chromosomes are

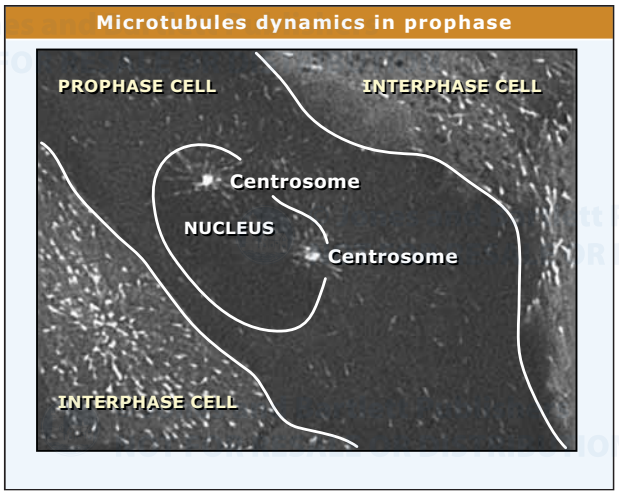

FIGURE 10.29 The first frame of a video (see CBIO: 10-0004) that shows live bird cells expressing a fluorescent version of a protein, EB1, a protein that binds to the tips of growing microtubules. Each white dot is the tip of a growing microtubule. The nucleus can be seen as a slightly darker region between and slightly to the left of the two centromeres. Photo courtesy of Patricia Wadsworth, University of Massachusetts, Amherst.

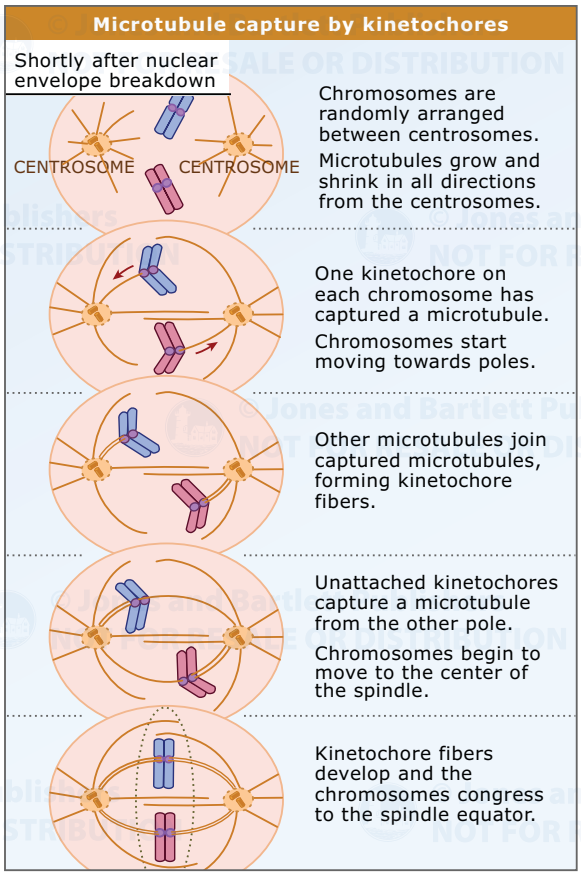

FIGURE 10.30 Dynamic microtubules search for kinetochores throughout the cell by growing and shrinking in random directions from the centrosomes. Microtubules that encounter a kinetochore are captured and stabilized. This search-and-capture mechanism of spindle assembly allows a spindle to be formed regardless of the shape of the cell or the positions of the chromosomes at the start.

arranged. Clearly the mechanism by which the microtubules find and attach to the kinetochores must be extremely flexible as well as exceptionally reliable.

These problems are solved by the dynamic behavior of the spindle's microtubules. Shortly before mitosis begins the two centrosomes become modified so that they are capable of nucleating many more microtubules than in interphase. At roughly the same time the microtubules become much more dynamic. Catastrophes occur more frequently and shrinking microtubules are rarely rescued and often depolymerize completely. These two changes create a situation in which large numbers of microtubules are constantly polymerizing in random directions from each of the two centrosomes, then depolymerizing and disappearing completely if they fail to be stabilized. Microtubules that are lost are replaced by others growing in other directions. The dynamic, searching behavior of the microtubules shortly before spindle assembly begins is shown in the video whose first frame is shown in FIGURE 10.29. The result of these dynamics is that after nuclear envelope breakdown the entire interior of the cell is continuously being probed by growing microtubule ends. Under these conditions it is simply a matter of time before every kinetochore is encountered by an astral microtubule. This search-and-capture mechanism ensures that all the kinetochores become attached to microtubules, and it allows a spindle to assemble regardless of how the chromosomes are positioned and oriented at the start of mitosis.

When a growing microtubule encounters a kinetochore it becomes trapped by molecular motors associated with the kinetochore's corona. In some cases the kinetochore attaches to the wall of the microtubule, while in others it becomes attached directly to its plus end. Under both conditions, *the kinetochore immediately begins to move rapidly poleward along the microtubule*, as illustrated in FIGURE 10.30 and the video whose first frame is shown in FIGURE 10.31. As a result of this motion, the chromosome is dragged toward the pole with the kinetochore leading the way. At some point during this process, kinetochores that initially attached to the side of a microtubule become attached to the plus ends of other astral microtubules. The poleward motion of a kinetochore as it attaches to the spindle orients it so that it now faces the pole. As the oriented kinetochore continues to move toward the pole, it

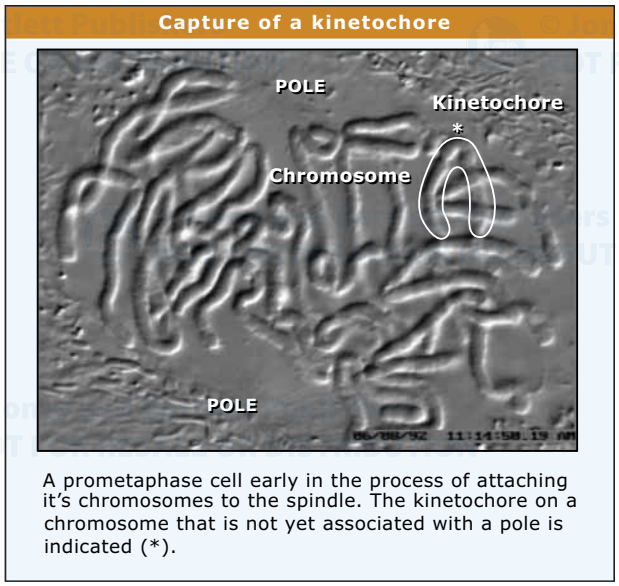

FIGURE 10.31 The first frame of a video (see CBIO: 10-0005) that depicts the attachment of a kinetochore to a microtubule and the subsequent movement of the chromosome to a pole. Photo © Conly Rieder, Wadsworth Center.

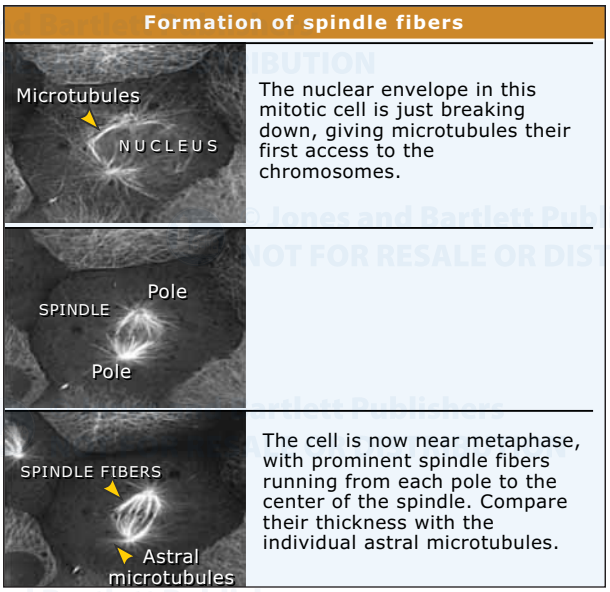

FIGURE 10.32 Frame from a video (see CBIO: 10-0006) that shows how microtubules attach to chromosomes and form spindle fibers. Photos courtesy of Patricia Wadsworth, University of Massachusetts, Amherst.

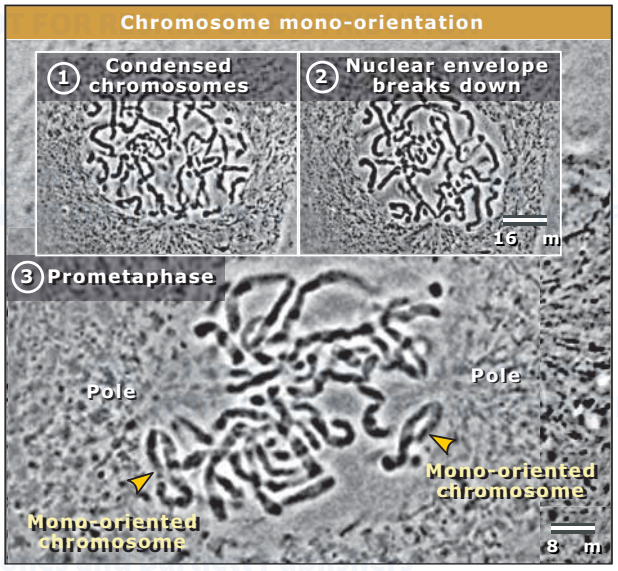

FIGURE 10.33 A sequence of light micrographs of a cell as its nuclear envelope breaks down and its chromosomes first become accessible to microtubules from the poles. Shortly after nuclear envelope breakdown several of the cell's chromosomes are clearly positioned near one of the poles and oriented with one kinetochore pointing toward it. The rest of the chromosomes are either still unattached or have already attached to both poles and moved to the middle of the spindle. Photos © Conly Rieder, Wadsworth Center.

captures more microtubules, beginning the formation of a kinetochore fiber. Because the kinetochore is facing the pole when the new microtubules are captured, most of them bind the kinetochore plate at their tips and terminate there. The gradual formation of kinetochore fibers early in mitosis is shown in FIGURE 10.32.

Owing to the random nature of the search-and-capture attachment mechanism, sister kinetochores rarely attach to the forming spindle simultaneously. After the first has attached, the chromosome is considered to be mono-oriented (see Figure 10.30, FIGURE 10.33, FIGURE 10.34, and FIGURE 10.35). The other kinetochore remains unattached until it captures a microtubule growing from the far pole. Once this occurs, the chromosome is bi-oriented and a kinetochore fiber connecting the chromosome with that pole develops (see Figure 10.30). *Bi-orientation is the only orientation that ensures that the two chromatids of a replicated chromosome will be distributed to opposite poles during anaphase.* Once a chromosome is bi-oriented, it begins to move toward the middle of the spindle, as shown in FIGURE 10.36. As it does so, the two kinetochores function differently: one must move toward the pole to which it is attached, requiring that the kinetochore fiber shorten, while the other must move away from its pole on an elongating fiber. Because bi-orientation is essential

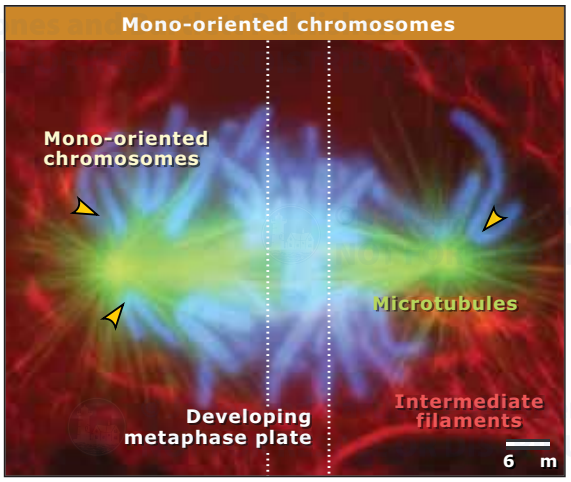

FIGURE 10.34 An immunofluorescence micrograph of a prometaphase cell. Many of its chromosomes have already attached to both poles and moved to the center of the spindle, but several—including those indicated with arrowheads—are still associated with only one pole. Note their clear "V" shape and proximity to the pole. Photo © Conly Rieder, Wadsworth Center.

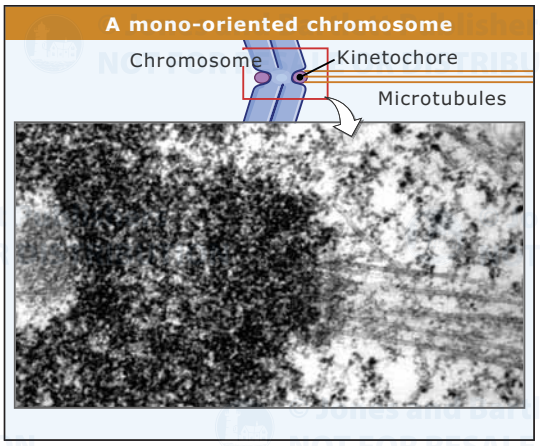

FIGURE 10.35 An electron micrograph of the centromere and kinetochores of a mono-oriented chromosome like the ones shown in Figure 10.33 and Figure 10.34. A bundle of parallel microtubules terminates in the kinetochore on the right; the other kinetochore has none. Photo © Conly Rieder, Wadsworth Center.

for the fidelity of chromosome distribution, cells have evolved a cell cycle checkpoint that monitors the proper attachment of sister kinetochores (discussed below).

Kinetochores change the properties of the microtubules they bind, and their influence is essential for converting the initial attachment of a chromosome to a single microtubule into the type of connection present in a fully formed mitotic spindle. The most important effect of interacting with a kinetochore is to make a microtubule much longer lived. Microtubules associated with a kinetochore have half-lives of about five minutes, while those in the rest of the spindle typically last less than one minute. The increased stability causes microtubules to accumulate at kinetochores, leading to the formation of kinetochore fibers. Even in kinetochore fibers, however, the microtubules are dynamic, with individual microtubules occasionally detaching from the kinetochore and being lost and new microtubules being incorporated.

The number of microtubules that ultimately bind to a kinetochore is influenced by the size of the kinetochore and the rate at which the microtubules attached to it turnover. The larger the kinetochore, the more microtubules it can engage at once. The kinetochores of higher animal cells generally have the capacity to bind between 20 and 40 microtubules, but the kinetochore fibers may have fewer because of the constant disassociation and reassociation of microtubules taking place.

What determines the rate with which kinetochore microtubules detach from the kinetochore? There is good evidence that it is due, in part, to the degree of tension between the kinetochore and its associated centromere. For example, if a fine needle is used to pull the centromere of a bi-oriented chromosome away from one of the poles as the spindle is forming, the number of kinetochore microtubules directed toward that pole increases. Clearly, *tension* somehow promotes the stability (and, thus, the accumulation) of microtubules at a kinetochore. One important implication of this is that it provides a means for selectively stabilizing the proper attachment of a chromosome to the spindle: sister kinetochores will be under maximum tension—and their kinetochore microtubules will be the most stable—when they are attached to and being pulled toward the opposite poles, *i.e., when they are properly oriented for a successful mitosis.*

10.12 Mistakes in kinetochore attachment are corrected

Key concepts

- Improper attachments often occur transiently as the chromosomes attach to the spindle.
- Improper attachments are unstable because they do not allow kinetochores to stabilize attached microtubules.
- Only the correct, bipolar attachment of a chromosome produces a stable kinetochore attachment.

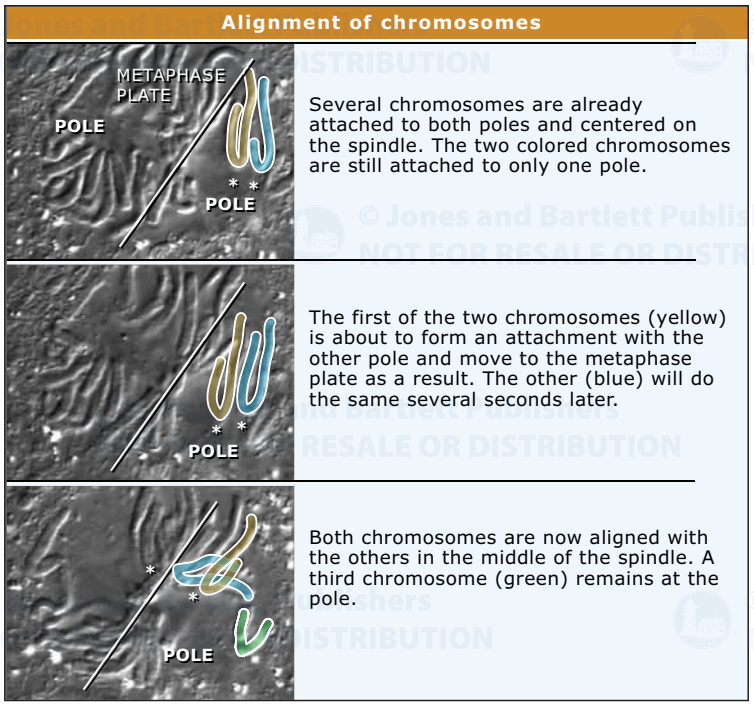

FIGURE 10.36 Frames from a video that shows the capture of microtubules by two mono-oriented chromosomes and the subsequent movement of the chromosomes to the center of the spindle. Photos © Conly Rieder, Wadsworth Center.

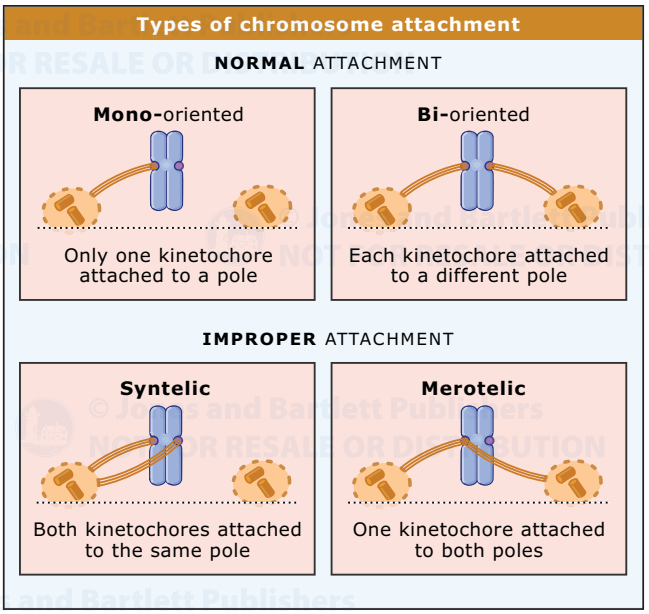

FIGURE 10.37 Bi-orientation is the only type of attachment that ensures that the chromosomes will be equally distributed to the two daughter cells; all chromosomes must be bi-oriented before anaphase begins. Mono-orientation is a normal intermediate on the way to bi-orientation. Syntelic and merotelic attachments often occur early in prometaphase but are corrected to bi-orientation before metaphase.

The sensitivity of the microtubule/kinetochore junction to tension plays an important role in correcting attachment errors that occur during spindle formation. The search and capture mechanism can produce two types of improper attachments, both of which occur during the course of a normal mitosis. A chromosome is syntelically (Greek: *syn* = same; *telos* = end) attached when both of its kinetochores are attached to the same pole, as shown in FIGURE 10.37. This condition usually occurs just after nuclear envelope breakdown, when the chromosomes face in random directions and may be much closer to one centrosome than the other, allowing sister kinetochores to simultaneously capture microtubules emanating from the same aster.

A single kinetochore may also become simultaneously attached to both poles (merotelic attachment; Greek: mero = part) (see Figure 10.37). Chromosomes containing one (or even two) merotelically oriented kinetochores move normally to the spindle equator and can pose a serious problem for the cell. If a merotelic attachment persists, the two chromatids separate as the cell enters anaphase, but the improperly attached chromatid remains stuck near the spindle equator. It then stays there until its attachment to one of the poles is broken, giving it a 50% chance of segregating to the same pole as its sister.

Both merotelic and syntelic attachments are normally corrected soon after they arise. In both types of improper attachment the kinetochore fiber microtubules attach to the kinetochore at a sharp angle, rather than perpendicularly. This distorts the kinetochore, which destabilizes the connection between it and the microtubules within the fiber. As a result, the microtubules detach faster and are replaced less frequently than in a proper attachment. Under this condition, the number of microtubules in a kinetochore fiber will sooner or later dwindle to zero, and one of the attachments will be broken. Depending on which type of improper attachment is involved, loss of an attachment either remedies the problem by creating a bi-oriented chromosome, or results in a chromosome that is attached to only one pole via a single kinetochore. This mono-oriented chromosome can then become bi-oriented by the usual mechanism.

The sensitivity of kinetochores to tension also plays a significant role in correcting im-

proper attachments. Improper attachments do not allow the kinetochores to face the poles, preventing the tension of a proper attachment from developing. Syntelic attachments (both kinetochores attached to the same pole) in particular are inherently unstable because there can be little tension exerted on the two kinetochores, certainly much less than if the chromosome were properly oriented and its centromere stretched between the two poles. This type of error is often corrected by the spontaneous loss of one of the connections because of lack of tension. Alternatively, the problem may be resolved when one of the two kinetochores acquires an additional attachment to the far pole, as shown in FIGURE 10.38. This causes the kinetochore to experience a sudden force in that direction, which distorts its structure and destabilizes the original attachment.

The intricacies of the attachment process demonstrate the complexity of kinetochores and the central role they play in mitosis. In fact, the separation of replicated chromosomes equivalently into two groups results directly from behaviors and functions associated with the kinetochores. In 1961, Mazia—one of the pioneers in thinking about the mechanisms of mitosis—emphasized their importance by stating that the kinetochore is "the only essential part of the chromosome so far as mitosis is concerned." He likened the rest of the chromosome to the "corpse at a funeral," in that it "provides the reason for the proceedings but does not take an active part in them." Although Mazia was right that the real goal of mitosis is to segregate sister kinetochores (the rest of the chromosome can be viewed largely as coming along for the ride), at the time he could not envision the extent to which these macromolecular assemblies are involved in directing this process.

In conclusion, kinetochore fibers attach the chromosome to the spindle and define the direction the chromosome will move. The equal segregation of chromosomes during mitosis can be related directly to the following facts: (1) each replicated chromosome contains two sister kinetochores; (2) these two kinetochores lie on opposite sides of the chromosome's primary constriction and face in opposite directions; (3) kinetochore fiber microtubules bind to the kinetochore only on its outer surface, which faces away from the chromosome; and (4) mechanisms exist to correct improper kinetochore-to-pole connections that would otherwise lead to aneuploidy.

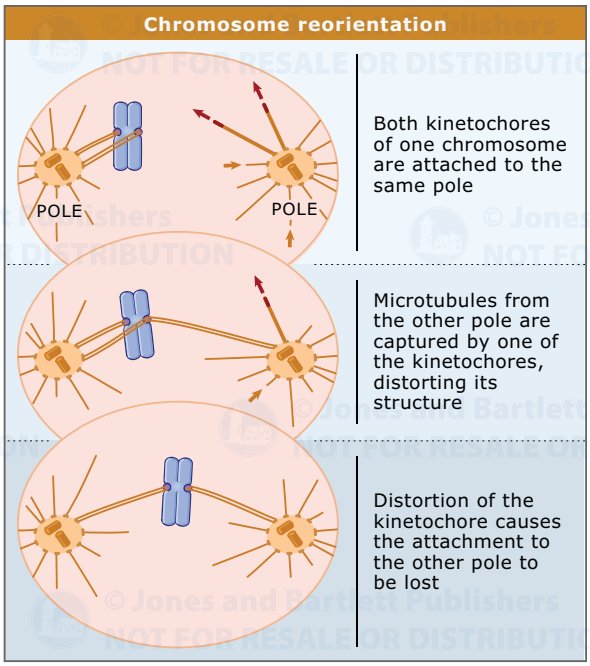

FIGURE 10.38 The ability of an improperly attached chromosome to reorient reliably depends on both the dynamics of microtubules during mitosis and the sensitivy of kinetochores to distortion. Only when the configuration at the bottom is achieved can kinetochore fibers develop and the chromosome be stably attached to the spindle.

10.13 Kinetochore fibers must both shorten and elongate to allow chromosomes to move

Key concepts

- Poleward forces are exerted on attached kinetochores during all stages of mitosis.
- Kinetochore fibers are anchored near the poles.
- Anchorage may depend on the spindle matrix, composed of the NuMA protein and a number of molecular motors.
- Kinetochore fibers change length by addition or loss of tubulin subunits at their ends.
- Both kinetochores and poles can remain attached to the ends of kinetochore fibers as the fibers change length.

The question "How do chromosomes move?" has been asked since the process of mitosis was discovered. In 1880, Flemming summarized the problem when he stated, "We do not know, in the movements or changes of position of the threads of a nuclear figure, whether the immediate causes lie within the threads themselves, outside of them, or both." (By "threads" Flemming was referring to the condensed chro-

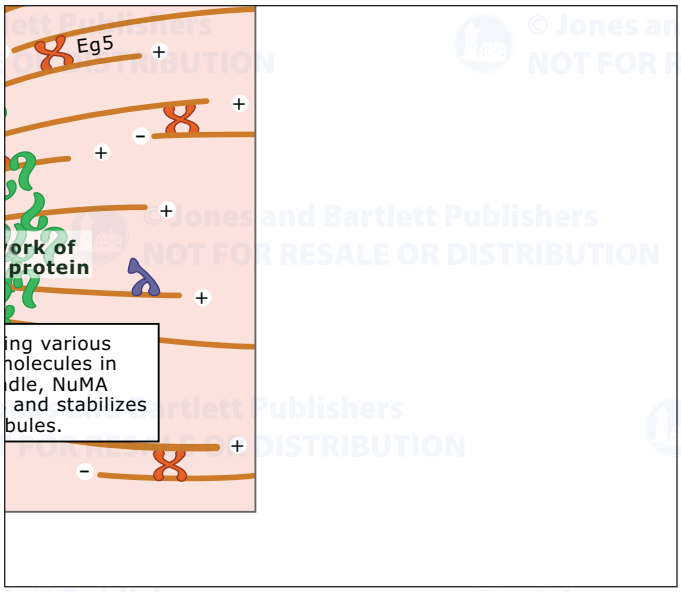

FIGURE 10.39 Within the spindle the NuMA protein forms a highly branched and crosslinked network—the matrix—that surrounds the microtubules and helps anchor and organize them at the poles. Motors associated with the matrix are likely to be responsible for microtubule dynamics that occur at the poles, including pulling kinetochore fibers poleward. The inset shows an immunofluorescence photograph of a metaphase cell with NuMA in red and the chromosomes in blue. Photo courtesy of Duane Compton, Dartmouth Medical School.

mosomes, and by "nuclear figure" he meant the mitotic spindle.)

We now know that a force directed toward the spindle pole begins to act on a kinetochore *as soon as it attaches to the spindle*. We also know *that this force acts during all stages of mitosis*, and *that the mechanism that generates it is the same throughout*. Thus, the poleward motion of a chromosome as it congresses during prometaphase occurs by the same mechanism that moves it toward a pole during anaphase.

In order for the chromosomes to move in response to forces exerted at their kinetochores, the kinetochore fibers must somehow be anchored. Without some form of anchorage, the chromosomes would remain in one place and reel in the microtubules rather than moving toward the poles along them. Micromanipulation experiments, in which individual chromosomes are tugged on by a very fine glass needle, reveal that it is difficult to pull a chromosome away from a pole but is relatively easy to move it from side to side. This implies that kinetochore fiber microtubules are most firmly anchored at their minus ends, near the poles of the spindle.

All of the microtubules in the spindle, including those in kinetochore fibers, are surrounded by the spindle matrix, as shown in FIGURE 10.39. The proteins of the spindle matrix may play a role in anchoring the kinetochore fibers. One of the major constituents of the matrix is the NuMA protein, which plays an important structural role in maintaining the integrity of the spindle. The concentration of NuMA within the spindle is related to microtubule density. As seen in Figure 10.39, microtubule density decreases gradually from the polar regions to the spindle equator, so that NuMA is particularly concentrated near the poles. The matrix also contains the microtubule-dependent motors Eg5 and HSET, both of which are kinesin-related proteins. HSET is unusual because, like cytoplasmic dynein, it moves toward the minus ends of spindle microtubules. As a result, HSET also accumulates in the spindle poles. The current model for how kinetochore fiber microtubules are anchored envisions that these motor proteins are attached to NuMA, which surrounds the microtubules near their minus ends. While bound to NuMA, the motors also interact with the walls of microtubules, creating a drag that resists their movement and serves as an effective anchor.

When a kinetochore moves away from its associated pole, as each does intermittently during congression, its fiber must elongate. Similarly, when it moves toward the pole, its fiber must shorten, as shown in FIGURE 10.40. *The elongation of kinetochore fiber microtubules occurs by the addition of tubulin subunits at the kinetochore. Shortening occurs by the loss of tubulin subunits at both the kinetochore and the poles.* In both cases, in order to remain continuously attached to its fiber the kinetochore must somehow hold onto the ends of the microtubules as they gain or lose subunits. *The polar attachment at the other end of the fiber must also be complex in order to allow for the loss of subunits observed during shortening.* The mechanisms for these remarkable behaviors at the ends of the fibers are not yet known. It is likely that the attachments at both ends depend heavily on motor proteins. The attachment at the kinetochore is thought to involve the kinesin-like protein CENP-E—a motor—as well as microtubule plus end-binding proteins and proteins in the centromere that promote the shortening of microtubule plus ends.

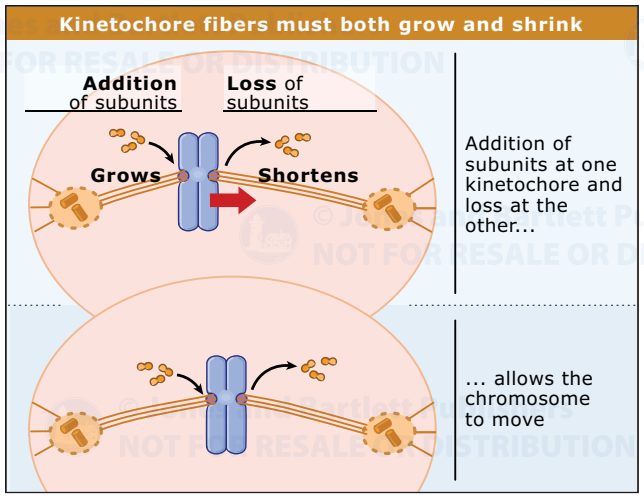

FIGURE 10.40 Movement of a bi-oriented chromosome within the spindle requires the simultaneous and coordinated growth and shortening of its two kinetochore fibers. The fibers change length by the gain or loss of tubulin subunits at the kinetochores.

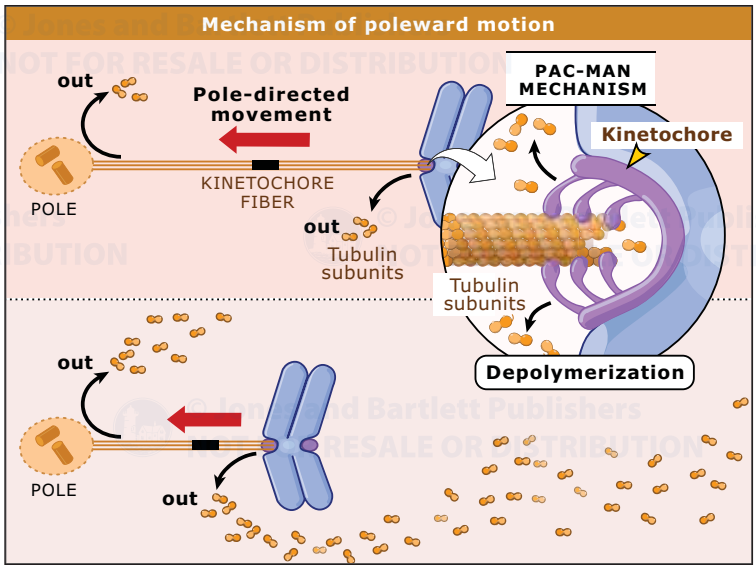

FIGURE 10.41 Motors at the kinetochore move it poleward along the microtubules within the kinetochore fiber. As the kinetochore moves, the microtubules depolymerize behind the motors. At the same time, other forces move the entire kinetochore fiber toward the pole, where subunits are also lost from the microtubules. Were a mark (shown here in black) made on the kinetochore fiber, both the distance between it and the kinetochore and the distance between it and the pole would decrease as the chromosome moved.

10.14 The force to move a chromosome toward a pole is produced by two mechanisms

Key concepts

- A kinetochore pulls the chromosome toward the pole but can move only as fast as the microtubules in the kinetochore fiber can shorten.
- Dynein at the kinetochore pulls a chromosome poleward on the ends of depolymerizing microtubules.
- Force generated along the sides of the kinetochore fiber also move the entire fiber poleward, pulling the chromosome behind it.

Once a kinetochore fiber is fully formed, a kinetochore pulls its associated chromosome toward the pole at about 1-2 µm/min. At this rate it takes between 10 and 20 minutes to cover 20 µm, or about half the length of a spindle. Theoretically, the force required to move an object the size of a large chromosome at this rate for 20 µm is only about 10^{-8} dynes. Surprisingly, the amount of energy that would be necessary to generate this force could be obtained by the hydrolysis of just 20 ATP molecules!

What actually occurs during mitosis, however, is much different. The force on a moving chromosome can be measured by determining how far it bends a fine glass needle placed in its path. Such experiments yield a surprising result: the maximum pole-directed force that the spindle can exert on a chromosome is ~10,000 times stronger than that theoretically needed to move it at the speeds typically observed during mitosis. Thus, *the rate of poleward motion is not determined by the amount of force acting on the kinetochore but rather by some other influence that keeps the velocity constant regardless of the force applied.* As an analogy, no matter how many horsepower the engine of a car can produce, the gear ratio limits how fast it can move; most cars cannot do 60 mph in first gear. In the case of the spindle, the limiting factor is the rate at which the microtubules in a kinetochore fiber can depolymerize: a kinetochore can move poleward only as fast as its microtubules can shorten.

The poleward motions of chromosomes are powered by two mechanisms. Both are present throughout mitosis, and they often act simultaneously, as shown in FIGURE 10.41. One mechanism involves the kinetochore; the other, its associated fiber. The contribution each makes varies depending on the type of cell. In vertebrate cells, motion results both from the activity of microtubule-dependent motors located at the kinetochore and from movement of the kinetochore fiber as a whole toward the pole.

In the "Pac-man" mechanism, motors in the kinetochore produce the force, moving the kine-

tochore poleward at the end of the kinetochore fiber as the microtubules in it depolymerize. Thus, as the kinetochore moves it can be viewed as "chewing up" the end of the fiber, giving the process its name. In the other mechanism, called the "traction fiber" mechanism, force is produced along the length of the kinetochore fiber. In this mechanism the whole kinetochore fiber slides toward the pole, pulling the kinetochore behind it.

The Pac-man mechanism for chromosome motion is powered by cytoplasmic dynein, a minus end-directed microtubule motor. Dynein located at the kinetochore actively pulls it poleward on the disassembling plus ends of its microtubules. During this motion, compression against the kinetochore, and/or catastrophe-promoting factors contained within its structure, induce the microtubule plus ends to disassemble. Dynein also plays a role during the initial attachment of kinetochores to the spindle, when they can move poleward along the side of a single microtubule at a velocity of more than 40 µm/min.

The traction fiber mechanism is based on the movement (flux) of tubulin subunits that occurs from one end of a kinetochore fiber to the other. Tubulin subunits are incorporated into each microtubule at the kinetochore and then migrate through it to the pole, where they are released (see Figure 10.13). As long as the incorporation of subunits at the kinetochore is equal to their loss at the pole, the kinetochore fiber remains a constant length and the kinetochore does not move. However, if the kinetochore stops incorporating tubulin subunits while they are still being removed at the pole, the kinetochore will be pulled toward the pole (see Figure 10.41). Thus, flux contributes a component of the force that moves a kinetochore and its attached chromosome toward a spindle pole.

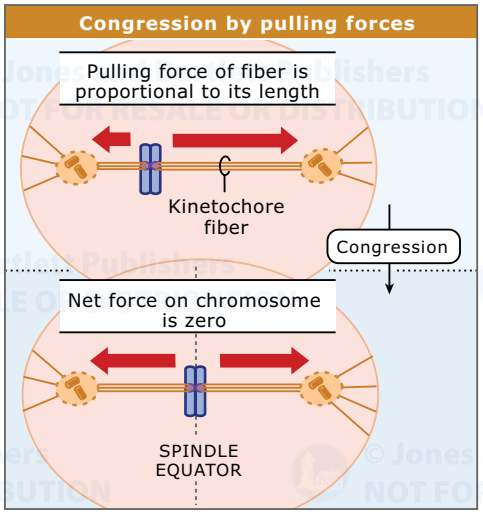

FIGURE 10.42 Ostergren's hypothesis for the mechanism of congression. A bi-oriented chromosome moves from left to right because the pulling force on each sister kinetochore is proportional to the length of its kinetochore fiber, with a longer fiber producing a greater pulling force. Here the length and direction of the red arrows indicate the magnitude and direction of the force. The chromosome moves until the two forces are balanced, which will occur in the middle of the spindle.

10.15 Congression involves pulling forces that act on the kinetochores

Key concepts

- The balance of several forces aligns the chromosomes at metaphase.
- Forces at both the kinetochores and along the arms of a chromosome participate.
- A plausible model suggests that poleward forces proportional to the length of each kinetochore fiber position the chromosomes in the center of the spindle.
- This mechanism may align the chromosomes in some types of cells.
- In many types of cells other forces must participate, including forces generated by the kinetochore and another that pushes chromosomes away from poles.

In 1945, Ostergren—another of the pioneers in thinking about the mechanisms of mitosis—offered an elegant explanation for congression. He proposed that "the equilibrium position of [a chromosome] strongly indicates that centromeres [kinetochores] are attracted to spindle poles by forces increasing in strength with an increasing distance between centromere and pole, and that each centromere is attracted only by that pole toward which it is turned." In other words, *the magnitude of the poleward pulling force that acts on each kinetochore would be proportional to the length of its associated kinetochore fiber*. In his view, a chromosome moves to the spindle equator because that is where its opposing kinetochore fibers are of equal length, and, thus, where the net force on the chromosome is zero, as shown in FIGURE 10.42.

Ostergren's "traction fiber" model draws support from the movement (flux) of tubulin subunits through kinetochore fibers. Flux suggests the presence of molecular motors anchored in the spindle matrix that surrounds the microtubules. These motors would exert a pole-directed pull on tubulin subunits along the entire length of the microtubules. The resulting movement of the microtubule toward the pole would be what we observe as flux. If such a mechanism does indeed exist, then the longer the microtubule the greater the pole-directed pulling force that will act on the kinetochore, just as Ostergren hypothesized. In this model, the kinetochore simply holds onto the end of a moving fiber and is pulled toward the spindle pole.

In some cells (such as plant cells and insect spermatocytes) microtubule subunit flux is the

only mechanism for moving the chromosomes toward a pole. In these systems, the traction fiber model remains the most feasible idea for how the chromosomes congress. In other cells, however, several of the predictions of the traction fiber model are not met and it is likely to be only a part of what is actually happening. In vertebrate cells, for example, the contribution of flux during the poleward motion of a chromosome accounts for only 30% of the chromosome's velocity. The remaining 70% is due to an activity associated with the kinetochore. At first glance these findings would appear to eliminate Ostergren's idea as a model for congression in vertebrates. However, the velocity of flux provides no information regarding the size of the force it produces. Thus, it is possible that the pole-directed force contributed by flux in vertebrates is much stronger than that produced by motors at the kinetochore.

The model must also be modified for vertebrates because it is clear that in vertebrate cells the forces that pull on kinetochores cannot be the only ones acting on the chromosomes. One observation that leads to this conclusion is the behavior of chromosomes attached to just one pole. Were the position of a chromosome determined solely by pulling forces at its kinetochores, as predicted by Ostergren's model, such mono-oriented chromosomes would be expected to move all the way to the pole. Instead, as FIGURE 10.43 shows, they often become stably positioned many micrometers away from it, suggesting the existence of a second force that pushes chromosomes away from poles.

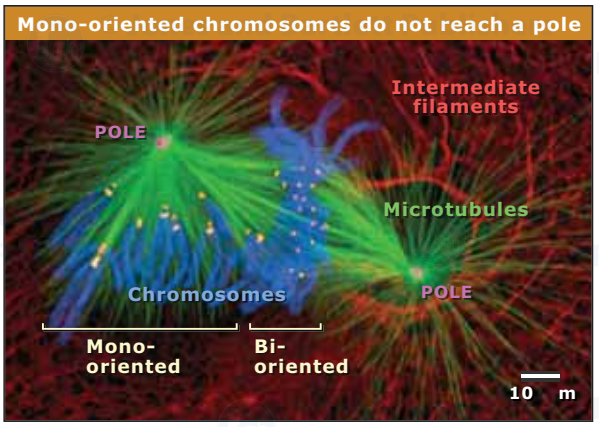

FIGURE 10.43 In this prometaphase cell, about half the chromosomes are already bi-oriented and aligned in the middle of the spindle. The others (at least 7) are still mono-oriented. Although clearly associated with only the pole on the left, all the mono-oriented chromosomes remain some distance from it. Kinetochores appear as yellow dots. Note how the pairs of dots on the bi-oriented chromosomes are all aligned parallel to the axis of the spindle and those on the mono-oriented chromosomes are oriented radially from the pole. Photo courtesy of Alexey Khodjakov, Wadsworth Center.

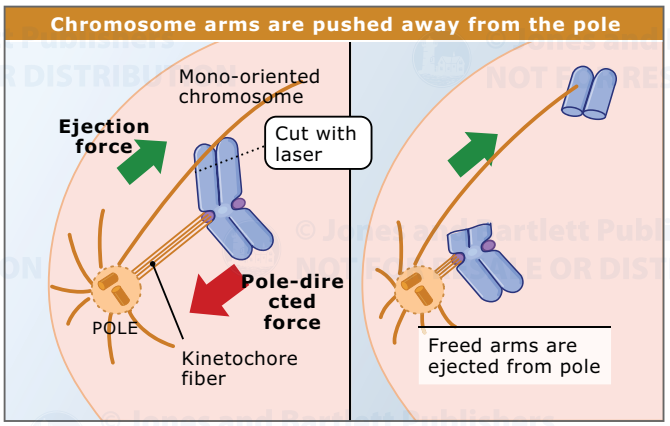

FIGURE 10.44 An experiment that demonstrates that chromosome arms experience a force that pushes them away from a pole. A mono-oriented chromosome is stably positioned at a distance from the pole to which it is attached. If the chromosome is cut, the piece without a kinetochore moves rapidly away from the pole while the the rest of the chromosome moves to a new stable position closer to the pole. Pole-directed forces at the kinetochore are thus opposed by ejection forces that act on the chromosome's arms.

10.16 Congression is also regulated by the forces that act along the chromosome arms and the activity of sister kinetochores

Key concepts

- Forces that act on the arms of chromosomes push them away from a pole.
- These forces arise from interactions between a chromosome's arms and spindle microtubules.
- Kinetochores can switch between active and passive states.
- Switching of sister kinetochores between the two states is coordinated.

In addition to the forces that act at the kinetochores, the arms of a mitotic chromosome also experience a force. This can be demonstrated experimentally by using a very finely focused laser beam to cut the arms of a mono-oriented chromosome away from its kinetochore, as shown in FIGURE 10.44. As illustrated in FIGURE 10.45, once freed, the arms are rapidly expelled from the polar region. This means that *during*

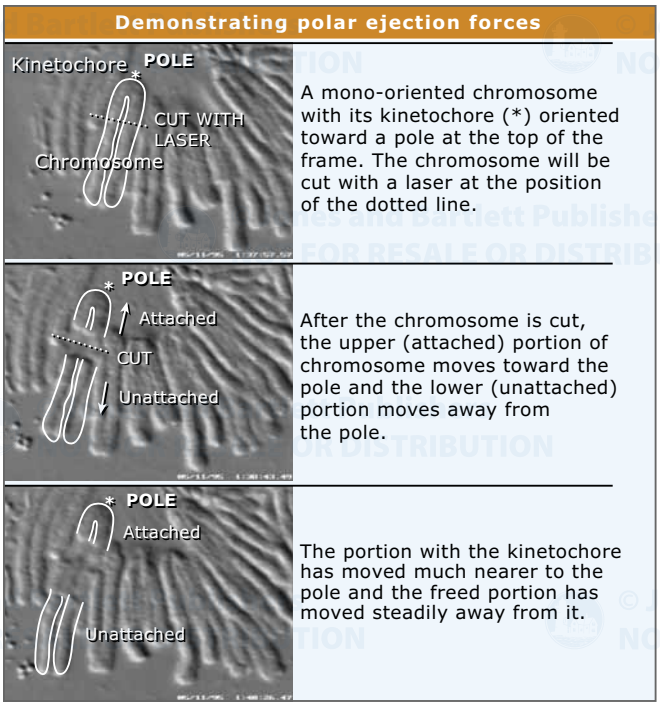

FIGURE 10.45 Mitotic chromosomes experience a force that pushes them away from a pole. Photos © Conly Rieder, Wadsworth Center.

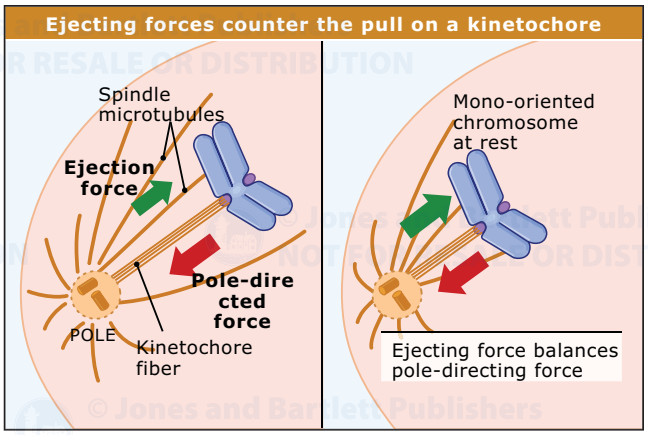

FIGURE 10.46 Schematic depicting the forces that work on a mono-oriented chromosome. Kinetochore-based forces (red arrow) pull the chromosome toward the pole while ejection forces (green arrow) push the chromosome away from the pole. The chromosome becomes stably positioned where these two forces are balanced (right). If one of the two forces is greater than the other, the chromosome will move toward or away from the pole (left).

mitosis chromosomes also experience forces that work to push them away from each pole. These ejection forces, called "polar winds," are generated in part by chromokinesins. These members of the kinesin family are located on the surface of the chromosome's arms, where they interact with spindle microtubules and move the chromosome away from the pole. Another, subtler mechanism also contributes to the ejection force. The ends of growing microtubules are capable of pushing objects as they grow, and the microtubules that are constantly growing away from each pole during mitosis may help push the chromosomes outward. Both of these mechanisms would produce a force that diminishes with distance from the pole as the microtubule density decreases. Under this condition, a chromosome attached to only one pole will come to rest at the position where the ejection forces of the polar winds are balanced by the pole-directed forces acting on the kinetochore, accounting for the inability of such chromosomes to move all the way to the pole, as seen in FIGURE 10.46.

The original traction fiber model also predicts that once a kinetochore is attached to the spindle it constantly experiences a pole-directed force. Were this the case, once attached to both poles chromosomes would move smoothly and continuously to the middle of the spindle and then remain motionless there until anaphase begins. This appears to be true for many types of cells, including those from plants and insects, in which the chromosomes do not move after they become positioned on the metaphase plate. However, in most cells congressed chromosomes constantly move small distances back and forth across the middle of the spindle, as seen in the movie whose first frame is shown in FIGURE 10.47.

These movements are caused by the kinetochores and reveal something important about how they work. In order for oscillations to occur, each kinetochore must have two different states that it can switch between. As illustrated in FIGURE 10.48, the switching of sister kinetochores between states must be coordinated in order for the chromosome to reverse direction. As a chromosome moves during an oscillation, the kinetochore that is moving toward its associated pole is producing or experiencing a pole-directed force. As the kinetochore moves, tubulin subunits must be lost from the microtubules in its kinetochore fiber. At the same time, the other kinetochore must be in a "neutral" state that allows it to be dragged away from its pole on the end of its kinetochore fiber, which is actively incorporating subunits in order to allow it to elongate. The chromosome reverses direction and a new oscillation begins when the two kinetochores exchange roles. The periodic switches in activity by a kinetochore are known as kinetochore directional instability, and the

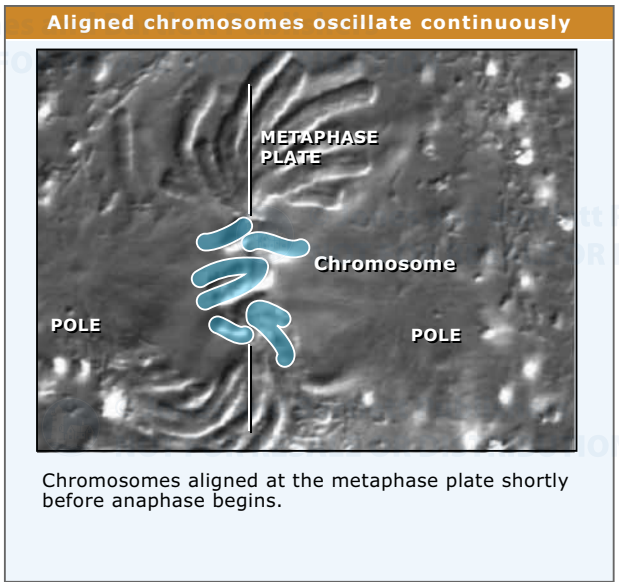

FIGURE 10.47 The first frame of a video (see CBIO: 10-0007) that shows that during metaphase, chromosomes do not remain stationary at the center of the cell but instead move back and forth constantly. Photo © Conly Rieder, Wadsworth Center.

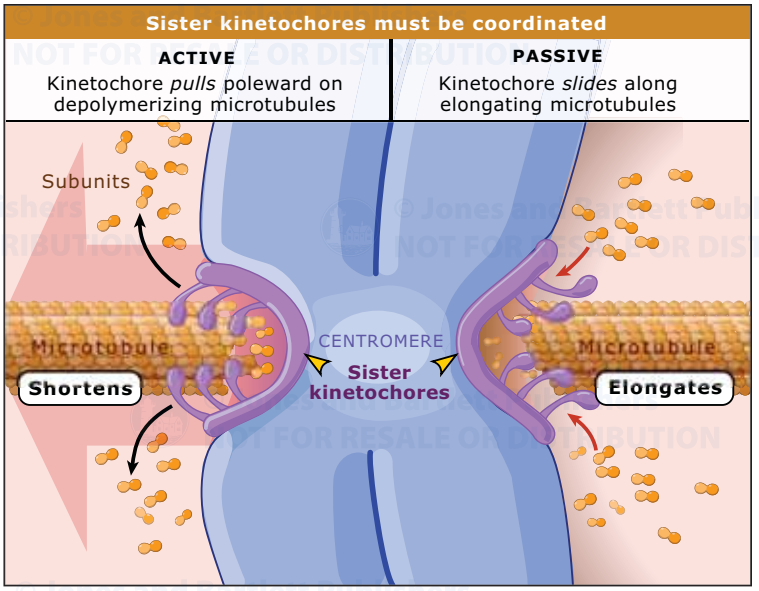

FIGURE 10.48 Once attached to the spindle, a kinetochore can exist in one of two activity states. One (left) allows it to move toward a pole on a shortening kinetochore fiber. The other (right) allows it to remain stationary or move away from its pole on an elongating fiber. The activities of the two sister kinetochores are somehow coordinated so that both kinetochores are rarely in the active state at the same time.

position of a chromosome on the spindle is heavily influenced by how the switching between sister kinetochores is coordinated. It is not known what causes a kinetochore to switch between the neutral and active states or what coordinates this activity between the sister kinetochores. Switching must be sensitive to the position of the chromosome relative to the spindle equator, however; otherwise the chromosomes could not remain reliably centered.

10.17 Kinetochores control the metaphase/anaphase transition

Key concepts

- A checkpoint prevents anaphase from beginning until all the kinetochores are attached to the mitotic spindle.
- Unattached kinetochores produce a signal that prevents anaphase from beginning.
- The checkpoint monitors the number of microtubules attached to a kinetochore.
- When all the kinetochores in a cell are properly attached the anaphase promoting complex (APC) is activated.
- Activation of the APC leads to the destruction of proteins that hold sister chromatids together.

The separation of chromatids at the start of anaphase provides visual evidence that a cell has undergone the metaphase-to-anaphase transition. With the exception of nuclear envelope breakdown, this is the most visually dramatic event in the cell cycle. As with nuclear envelope breakdown, this event is also irreversible.

If sister chromatids separated before all the chromosomes are attached to both poles of the spindle, aneuploidy would be a frequent event. To prevent this, a cell cycle checkpoint has evolved that monitors the attachment of kinetochores to the spindle during mitosis. Unattached or weakly attached kinetochores, which would indicate an incompletely assembled spindle, produce a signal that delays the onset of anaphase until the problem is corrected—a "wait anaphase" signal (see *11 Cell cycle regulation*). We know this because anaphase normally will not occur as long as a cell contains even a single mono-oriented chromosome but will begin shortly after the unattached kinetochore on that chromosome is destroyed with a laser microbeam, as shown in FIGURE 10.49. The signal is automatically produced as soon as the nuclear envelope breaks down because the kinetochores of the newly exposed chromosomes are all unattached. Therefore, this signal is continuously present until the last kinetochore attaches to the spindle.

This checkpoint, called the kinetochore attachment or spindle assembly checkpoint, ensures that the chromosomes are properly

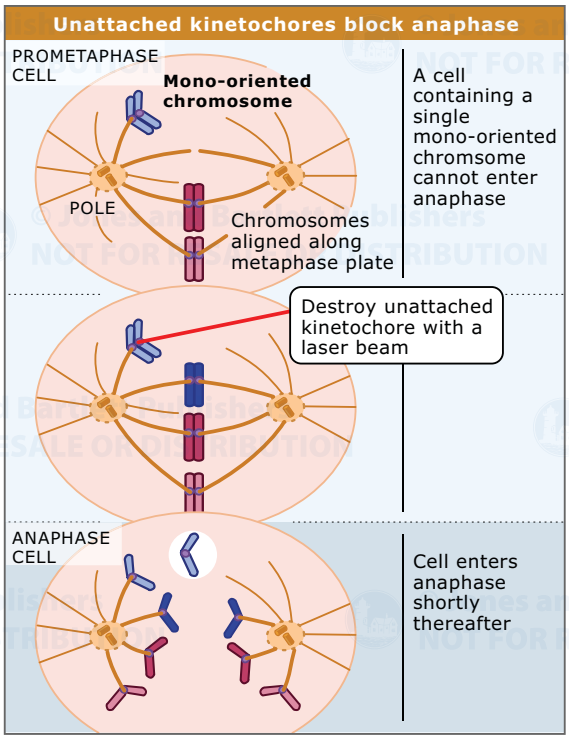

FIGURE 10.49 An experiment demonstrating that an unattached kinetochore produces a signal that prevents anaphase from beginning. The cell is stuck in prometaphase at the top. After an extremely fine laser beam (red) is used to inactivate the components of its one unattached kinetochore, anaphase soon begins.

segregated in the great majority of cases. However, it cannot detect when a single kinetochore is simultaneously attached to both poles (i.e., merotelically oriented). The inability of the checkpoint to detect this type of malorientation means that anaphase sometimes occurs before the problem can be corrected. Although mistakes in the segregation of chromosomes are normally rare, they do occur approximately once every 10,000 divisions. Current evidence suggests that merotelic attachment is a leading cause of aneuploidy in tissue cells.

The spindle assembly checkpoint works by monitoring how many microtubules are attached to a kinetochore. When too few microtubules are present, the kinetochore produces the wait anaphase signal. This signal is turned off once the number of microtubules acquired by the kinetochore increases above a critical threshold. Since tension on a kinetochore stabilizes and promotes the accumulation of microtubules by the kinetochore, the checkpoint is sensitive to whether a kinetochore is under tension. The greatest tension exists when sister kinetochores are attached to opposite poles, so by monitoring the number of microtubules at the kinetochores the checkpoint is able to indirectly assess whether all the chromosomes are properly attached to the spindle.

When spindle microtubules are disrupted with drugs, the spindle assembly checkpoint remains active for a prolonged period. The persistence of the signal in the presence of such drugs was used as a screen to isolate yeast mutants that are defective in genes needed for the checkpoint. The genes that were discovered code for three Mad (*m*itosis *a*rrest *d*eficient) and three Bub (*b*udding *u*ninhibited by *b*enzamidazole) proteins. These proteins have counterparts (homologs) in vertebrate cells, including those of humans. When the activity of any Bub or Mad protein is inhibited, the checkpoint is overridden, and anaphase occurs shortly thereafter. During mitosis, several of these proteins, including BubR1 and Mad2, constantly cycle on and off unattached kinetochores but are not found on attached kinetochores.

The critical event that initiates anaphase, which in turn allows sister chromatids to separate, is the activation of a large macromolecular assembly known as the anaphase-promoting complex. The job of the anaphase-promoting complex is to target selected proteins for destruction, and it does so by attaching a string of ubiquitin molecules to them. This tag then allows the protein to be recognized and degraded by the cell's proteolytic machinery (proteosomes). The anaphase-promoting complex cannot work by itself, however. Rather, a cofactor is required to specify which proteins are to be targeted for destruction and at what time. Anaphase onset requires that the anaphase-promoting complex be activated by the Cdc20 cofactor. *The spindle assembly checkpoint works by inhibiting the activity of Cdc20, which, in turn, prevents the anaphase-promoting complex from targeting the protein* securin *for destruction*. The destruction of securin leads to the destruction of glue proteins on the chromosomes that actually hold the replicated chromatids together.

Exactly how the kinetochore and the components of the checkpoint pathway influence Cdc20 is not yet clear. The question is how the signal is transmitted from the kinetochore into the spindle, where the anaphase-promoting complexes are found. One view is that complexes composed of Mad2, BubR1, and Cdc20 are formed at the kinetochore and then released. Another possibility is that unattached kinetochores bind and activate one or more of the

checkpoint proteins and then release the activated proteins into the spindle where they form complexes with Cdc20 that prevent it from activating the APC. Although it is not yet clear where complexes between Cdc20 and the components of the checkpoint pathway are formed, it is clear that they are formed continuously as long as an unattached kinetochore exists but are short lived so that inhibition of the APC is quickly relieved once the last kinetochore is attached. How the wait-anaphase signal emitted by the last unattached kinetochore is amplified so that it is heard throughout the spindle remains to be discovered.

10.18 Anaphase has two phases

Key concepts

- Destroying the connections between sister chromatids allows them to begin moving toward opposite poles.
- Movement occurs because pulling forces that act on sister kinetochores throughout mitosis no longer oppose one another.
- Elongation of the mitotic spindle during anaphase increases the distance between the separating chromosomes.
- Spindle elongation is caused by both pushing forces that act on midzone microtubules and pulling forces that act on astral microtubules.

After the last kinetochore has attached to the spindle, there is a lag period before the chromatids separate. During this period, securin and other proteins are degraded. Once chromatid separation begins, the process is completed throughout the cell within several minutes. Normally, sister chromatids separate first in the centromere region, because that is where the opposing pole-directed forces act on the chromosome. After this region separates, the sister chromatids are then "peeled" apart as they move toward their respective poles, as shown in FIGURE 10.50.

The process by which the two sets of newly separated chromosomes move closer to the poles is called anaphase A. This is to distinguish it from anaphase B, the process by which the poles themselves move apart. Anaphase's A and B are illustrated in FIGURE 10.51. The two processes are not different temporal stages of anaphase but are instead two independent mechanisms that work simultaneously to separate the chromosomes.

Although the chromosomes abruptly begin moving toward the poles at the start of anaphase, the force-producing mechanism that moves them is not suddenly turned on at the metaphase-anaphase transition. This is evident from an experiment in which a laser beam is used to destroy one kinetochore of a bi-oriented chromosome during prometaphase, well before

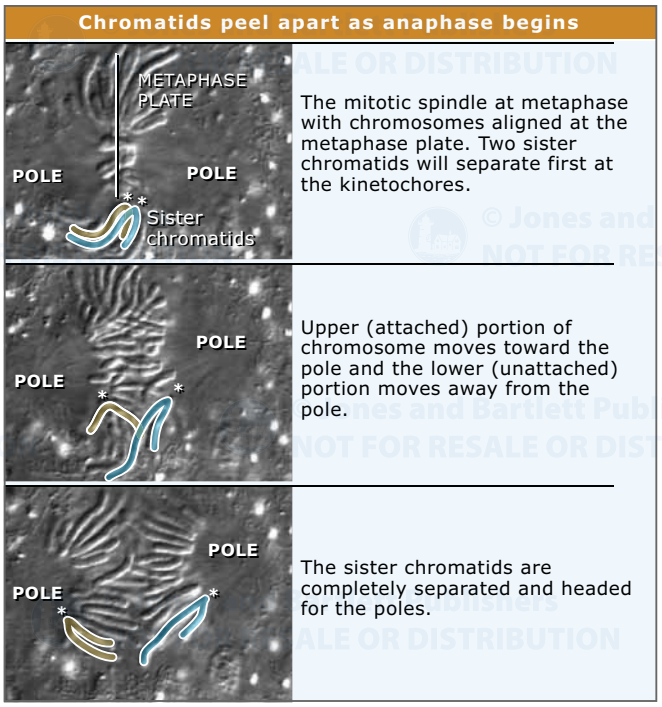

FIGURE 10.50 Frames from a movie (see CBIO: 10-0008) that follows the chromosomes as they are pulled apart and away from the metaphase plate. Photos © Conly Rieder, Wadsworth Center.

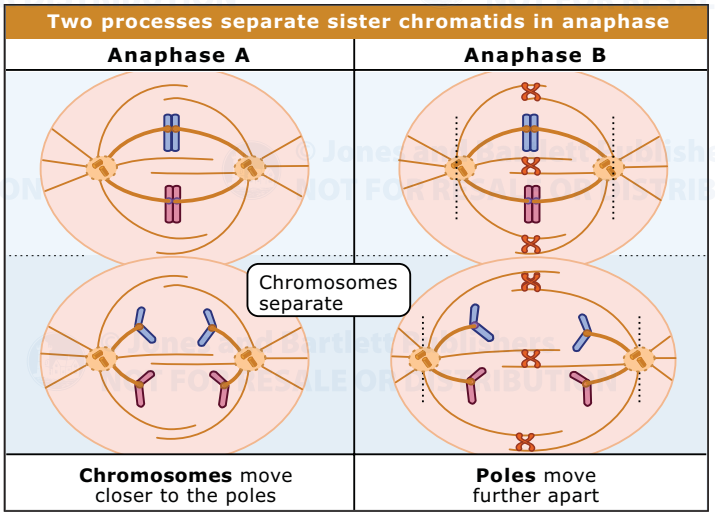

FIGURE 10.51 As the chromosomes move to the poles (anaphase A), the poles themselves move farther apart (anaphase B), increasing the separation between the two groups of chromosomes. The poles are moved by pulling forces on the astral microtubules and motors in the center of the spindle that slide overlapping microtubules past one another. Together, anaphases A and B ensure that the two new nuclei are far enough apart so that the cell can be reliably divided between them.

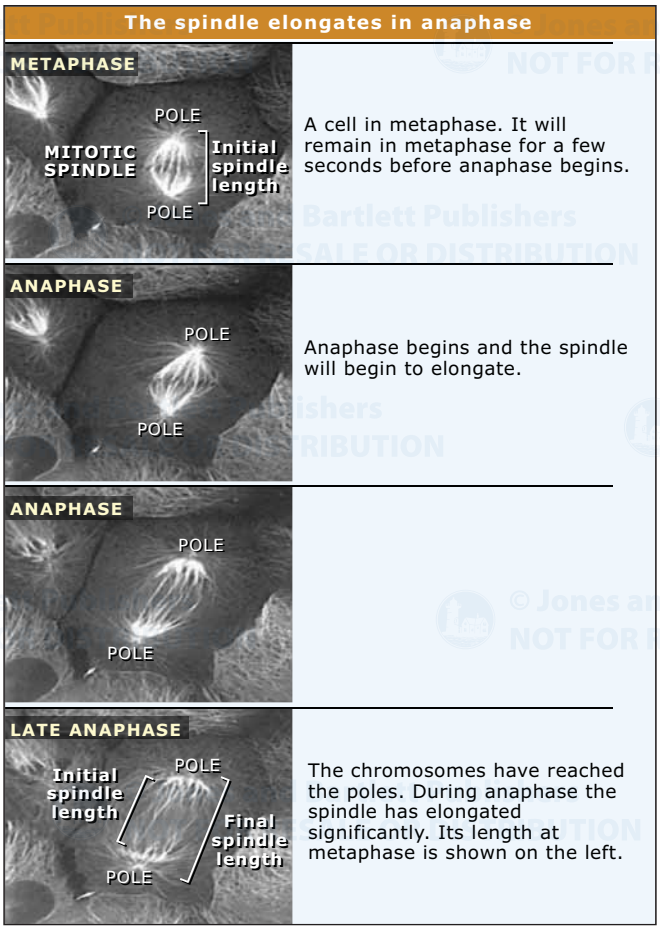

FIGURE 10.52 Frames from a video (see CBIO: 10-0009) that depicts the lengthening of the spindle during anaphase. Photos courtesy of Patricia Wadsworth, University of Massachusetts, Amherst.

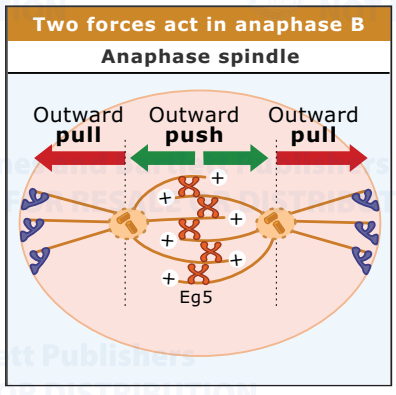

FIGURE 10.53 Two mechanisms are used to move the spindle poles apart as the spindle elongates in anaphase B. Bifunctional kinesin-like molecular motors in the middle of the spindle (orange) push on microtubules of opposing polarity. At the same time, cytoplasmic dynein (purple) anchored at the cell cortex pulls on astral microtubules.

the chromatids normally separate. Freed of its connection to one of the poles, the chromosome immediately moves toward the other pole in exactly the same way as an anaphase chromosome. Thus, the same mechanism(s) that moves a chromosome toward a pole during spindle formation and congression also moves it toward a pole during anaphase A. Poleward forces on the kinetochores are continuously present throughout mitosis. The only difference during anaphase is that the forces acting on sister kinetochores no longer oppose one another and can now act independently. The result is that the chromatids abruptly begin moving poleward as soon as they are separated. As in movements during earlier stages of mitosis, in vertebrates this poleward motion is powered by both activity at the kinetochore and microtubule flux.

As the two separating groups of chromatids move toward their respective poles during anaphase A, the poles themselves move further apart, as shown in FIGURE 10.52. This process of spindle elongation is anaphase B. *Anaphase B increases the separation between the two groups of chromatids*, ensuring that the furrow that will later divide the cytoplasm and create two new cells occurs between the two reforming nuclei. In some organisms, anaphase B begins only after the completion of anaphase A. However, in vertebrates and most other cells, the distance between the spindle poles begins to increase as soon as the chromatids disjoin, so that the two phases occur at the same time. In general, the extent that the spindle elongates varies widely, even among adjacent cells in the same culture. Some of the variation is due to shape; the spindle tends to elongate more in long, rectangular cells than in smaller, rounder ones.

As illustrated in FIGURE 10.53, several mechanisms operate to separate the poles during anaphase B. In many single-celled organisms—like yeast, diatoms, and fungi—the process is powered by forces produced in the spindle midzone, between the two separating groups of chromosomes. Within this zone microtubules that originate at the two poles overlap and kinesin-like proteins bind and cross-link neighboring microtubules of opposite polarity. As these molecular motors move toward microtubule plus ends, they slide adjacent microtubules past one another, pushing each in the direction of the pole to which it is attached. The spindle elongates as a result. While this is happening the interzonal microtubules also grow at their plus ends so that the zone of overlap is

maintained. The growth of these microtubules determines how much the entire spindle will elongate.

As with the forces that drive anaphase A, these pushing forces are also present during the earlier stages of mitosis. Before anaphase, however, they are opposed by other forces generated within the spindle that work to pull the poles closer together. These opposing forces are generated partly by microtubule minus end motors that also link adjacent microtubules of opposite polarity. They are also produced by the sister kinetochores on bi-oriented chromosomes, which are constantly working to pull the poles toward the metaphase plate. When the chromatids finally separate at the onset of anaphase, this balance of forces is disrupted as the forces that act to pull the poles together are weakened. As a result, the pushing forces between the poles dominate and the poles are pushed apart.

How much this "pushing" mechanism contributes to spindle elongation in vertebrate cells is controversial. This is because in these cells the minus ends of the spindle's non-kinetochore microtubules become detached from the poles as they separate during anaphase B. Thus, by the middle of anaphase the spindle poles in vertebrate cells are not being pushed apart but rather are being pulled apart (see Figure 10.53). The pulling forces are generated by interactions between the spindle's astral microtubules, which remain connected to the poles during anaphase, and cytoplasmic dynein anchored in the cell periphery (i.e., within the cell cortex). Dynein molecules anchored in the cortex "reel in" astral microtubules, pulling the two poles apart.

10.19 Changes occur during telophase that lead the cell out of the mitotic state

Key concepts

- The same cell cycle controls that initiate anaphase also initiate events that lead to cytokinesis and prepare the cell to return to interphase.
- Inactivation of CDK1 by destruction of cyclin B reverses the changes that drove the cell into mitosis.
- Destruction of cyclin B begins when the spindle assembly checkpoint is satisfied, but a lag prevents telophase from beginning before the chromosomes have separated.

After the chromosomes have been separated the cell must start cytokinesis and ultimately leave mitosis. The coordination of chromosome separation with these events is due to the same checkpoint that controls the transition from metaphase to anaphase. In addition to delaying the separation of the chromatids, the checkpoint delays the activation of biochemical pathways that cause the cell to undergo cytokinesis and drive it out of mitosis. Once those pathways are activated, however, they cannot be stopped. As a result, once a cell enters anaphase it is committed to leaving mitosis.

As with chromatid disjunction, the pathway that causes a cell to leave mitosis is controlled by the anaphase promoting complex. Once the pathway is activated it induces the cell to enter telophase, the last stage of mitosis. During telophase the chromosomes within each anaphase group swell and decondense, while a new nuclear envelope is formed around their periphery (see Figure 10.1). At the same time, the spindle disassembles and the microtubules in the asters grow longer, as shown in FIGURE 10.54, and a new microtubule-based structure

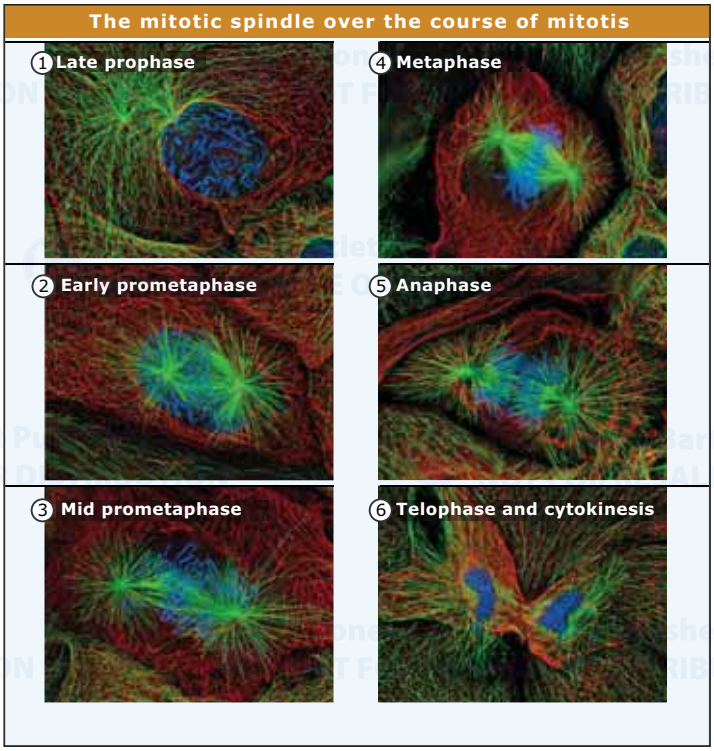

FIGURE 10.54 The immunofluorescence micrographs show microtubules (green), keratin filaments (red), and the chromosomes (blue) at successive stages of mitosis. Photos © Conly Rieder and Alexey Khodjakov, Wadsworth Center.

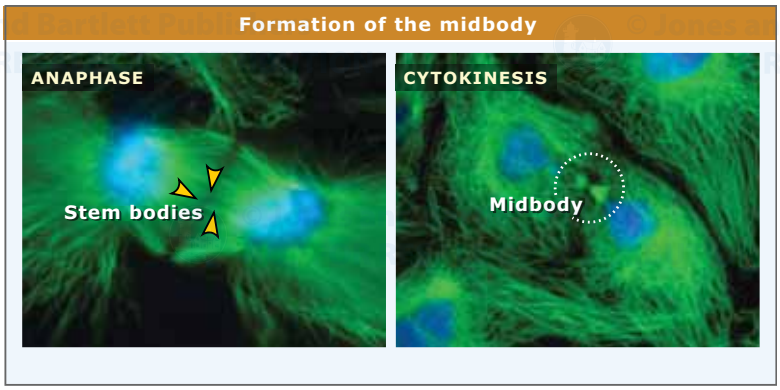

FIGURE 10.55 Microtubules are in green and DNA in blue. In the anaphase cell many small bundles of microtubules—stem bodies (several are indicated by arrowheads)—extend between the two recently separated groups of chromosomes. By the end of cytokinesis, the stem bodies have coalesced to form the midbody, a single structure between the two nuclei. The center of the midbody and each stem body is dark because it has a specialized structure that the green dye used to stain the microtubules cannot penetrate. The small nonstaining region in the middle of each stem body produces an apparent dark line across the middle of the anaphase cell. Photos © Conly Rieder, Wadsworth Center.

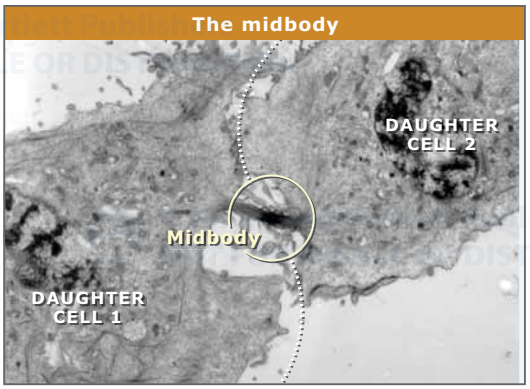

FIGURE 10.56 An electron micrograph showing a midbody. Cytokinesis is almost complete and only a thin bridge of cytoplasm occupied by the midbody connects the two daughter cells. The dotted line indicates the approximate boundary between the two cells. Photo © Conly Rieder, Wadsworth Center.

called the midbody forms in the area between the two separated groups of chromosomes, as illustrated in FIGURE 10.55 and FIGURE 10.56. As these events are occurring within the cell other events at its surface prepare it for cytokinesis.

Telophase is triggered by destruction of the cyclin B subunit that activates CDK1, the kinase that creates the mitotic state. We know this because when a cell is filled with a type of cyclin B that cannot be destroyed the chromatids disjoin normally and complete anaphase A and B. However, the anaphase spindle does not disassemble, a midbody is not formed, the chromosomes remain as individuals, nuclei do not re-form, and cytokinesis does not occur.

Cells are driven out of mitosis by a reversal of the events that drove them into it. As discussed earlier, cells are driven into mitosis by the sudden activation of the cyclin B/CDK1 kinase. This enzyme then phosphorylates target proteins, which changes their activities and/or locations. The nuclear envelope and the cell's endomembrane system, including the Golgi complex and the endoplasmic reticulum, are induced to break up into small vesicles. The properties of the centrosomes and their associated microtubule arrays are also changed in ways that promote the formation of the spindle. When the kinase is inactivated during anaphase by proteolysis of its cyclin B regulatory subunit, the proteins that it phosphorylated in order to induce these changes become dephosphorylated. As this occurs, the events that produce the mitotic state are gradually reversed.

The degradation of cyclin B usually starts soon after the spindle-assembly checkpoint is satisfied. However, the events of telophase do not normally occur until anaphase A is completed about 10-15 minutes later. The practical reasons for this delay are obvious: it would be potentially disastrous for the cell if the spindle were to disassemble and the nuclei to reform before the two groups of chromosomes were a significant distance apart. To create the delay a lag is somehow built into the series of biochemical events that follow the destruction of cyclin and lead to the changes in cellular structures that it induces.

10.20 During cytokinesis, the cytoplasm is partitioned to form two new daughter cells

Key concepts

- The two newly formed nuclei that are the products of karyokinesis are separated into individual cells by the process of cytokinesis.
- Cytokinesis involves two new cytoskeletal structures: the midbody and the contractile ring.
- The mitotic spindle, the midbody, and the contractile ring are all highly coordinated with one another.
- Cytokinesis has three stages: definition of the plane of cleavage, ingression of the cleavage furrow, and separation of the two new cells.

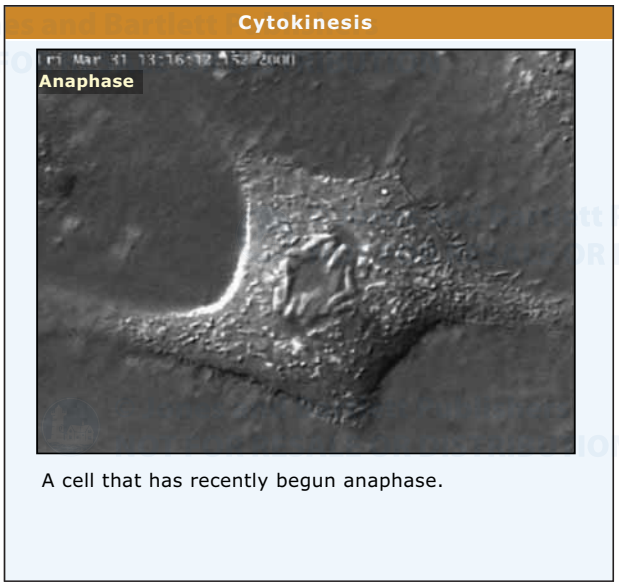

FIGURE 10.57 The first frames of a video (see CBIO: 10-0010), which shows that cytokinesis begins when the chromosomes have finished separating. The process divides the cell in two by a deep furrow that forms between them. Photo © Conly Rieder and Alexey Khodjakov, Wadsworth Center.

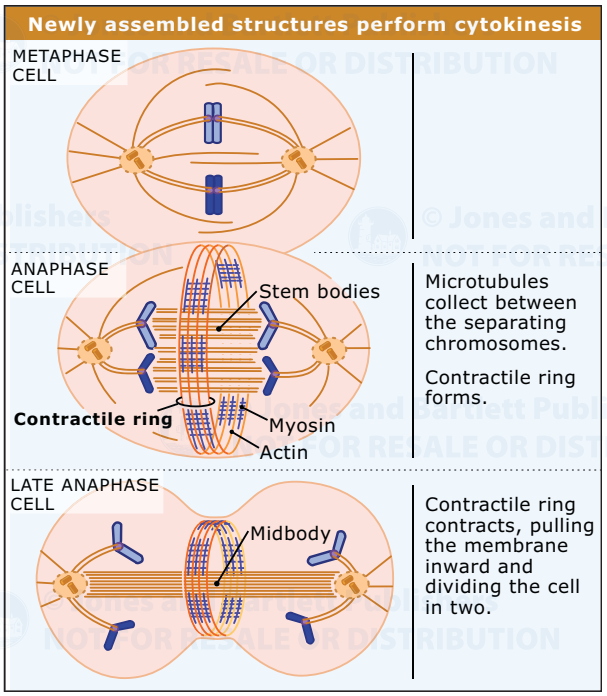

FIGURE 10.58 The midbody is a large microtubule structure that forms from bundles of microtubules that collect between the separating chromosomes during anaphase. At the same time the contractile ring, a tight band of actin and myosin filaments, forms around the cell immediately beneath its plasma membrane. The midbody directs the placement of the ring, which will divide the cell into two.

After a cell separates its chromosomes, it must divide. Animal cells accomplish division by constricting between the two newly separated sets of chromosomes. Near the end of anaphase B, the surface of the cell begins to constrict in the same plane that the chromosomes occupied at metaphase. Over the next 10-15 minutes the cell is pinched in two in the process known as cytokinesis or cleavage, as seen in the video whose first frame is shown in FIGURE 10.57. Cytokinesis represents the last phase of mitosis. It occurs as the cell is exiting the mitotic state and requires that the cyclin B/CDK1 kinase be inactivated.

Like the separation of the chromosomes, cytokinesis not only requires the presence of the mitotic spindle but also involves the formation of two new structures, the midbody and the contractile ring, as shown in FIGURE 10.58. The midbody is formed during anaphase as microtubules from the spindle are reorganized into a large bundle of parallel microtubules that extend between the two groups of separating chromosomes. It forms gradually as numerous independent small bundles coalesce, as illustrated in FIGURE 10.59. The contractile ring is composed of actin filaments bundled together to form a tight band immediately beneath the plasma membrane. Bipolar myosin filaments similar to those in muscle connect them. Both the midbody and the contractile ring contain many proteins in addition to those that form these principal structural features.

The contractile ring is responsible for powering the constriction process that divides a cell in two. As its name implies, the ring is capable of contraction, driven by interactions between the actin and myosin within it. Because it is attached to the plasma membrane, the ring works in a manner similar to that of a purse string: contraction decreases its diameter, gradually decreasing the size of the opening between the two halves of the cell. Because contraction at the wrong place or time would be catastrophic, the formation and function of the ring depend on interactions with the other two cytoskeletal elements present during cytokinesis. The position of the spindle determines where the midbody forms, which in turn defines the site where the contractile ring assembles. *This sequence ensures that the ring forms between the separated chromosomes.* The chromosomes themselves play a role in the timing of contraction; as anaphase begins they contribute factors to the ring that

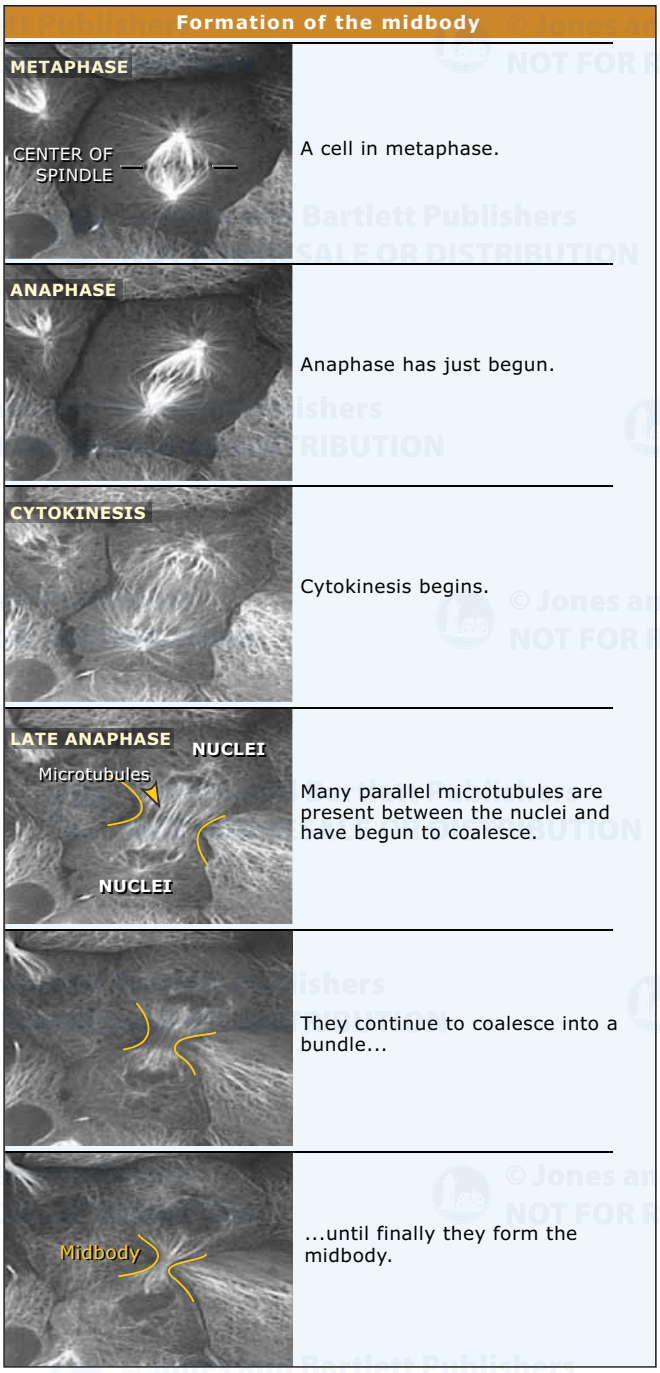

FIGURE 10.59 Frames from a video (see CBIO: 10-0011) that shows the midbody form during anaphase. Photos courtesy of Patricia Wadsworth, University of Massachusetts, Amherst.

are apparently required for it to function. The failure of any of these events prevents cytokinesis and results in a cell with two nuclei. Such binucleate cells exist in some human tissues (e.g. lung, liver), but they rarely divide again.

Like the separation of the chromosomes, the process of cytokinesis can be subdivided into several sequential stages, each of which is defined by one or more recognizable events. Among them there is only one point of no return, which occurs at the very end of the process. Thus, *cleavage is fully reversible until the daughter cells are separate entities.*

During the first stage of cytokinesis, which occurs shortly after anaphase begins, the location within the cortex of the cell where the contractile ring will be assembled is determined, as shown in FIGURE 10.60. This *defines the plane* in which the cell will divide. Next, the contractile ring is assembled at this site. Even as the ring is forming it starts to contract, dimpling the surface of the cell and beginning the "ingression" stage of cytokinesis. As the ring continues to contract the dimple becomes a deepening furrow that runs all the way around the cell. This cleavage furrow eventually constricts the cell into two lobes connected only by a thin bridge of cytoplasm containing little other than the midbody. Following this, during the final separation or "abscission" stage, the cell passes a point of no return and the cytoplasmic bridge breaks, creating two independent daughter cells.

10.21 Formation of the contractile ring requires the spindle and stem bodies

Key concepts

- The location of the mitotic spindle determines where the contractile ring forms.
- The mitotic spindle is positioned by interactions between its astral microtubules and the cortex of the cell.
- Bundles of parallel microtubules called stem bodies form between the two separating groups of chromosomes in anaphase.
- As anaphase progresses the stem bodies coalesce into one large bundle called the midbody.
- Stem bodies signal to the cortex to cause the formation of the contractile ring.

Where the contractile ring forms is determined by an interaction between the mitotic spindle and the cortex of the cell. Early in anaphase the entire cortex is competent to support the formation of a contractile ring and furrow, but only a small area of it later does. The significance of the spindle in determining which area is selected is demonstrated by experiments in which an anaphase cell is manipulated to move its spindle to a new location. If the spindle is moved

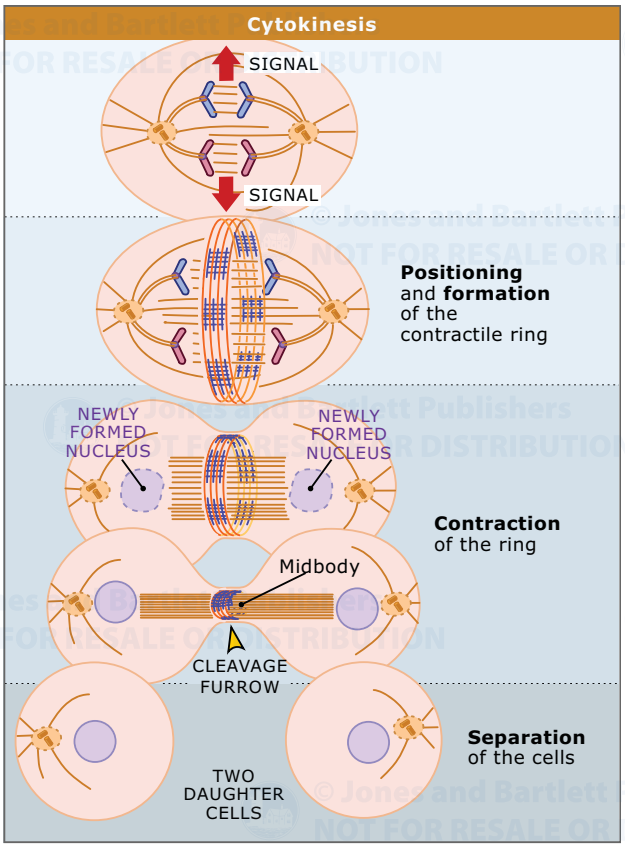

FIGURE 10.60 The stages of cytokinesis. Signals from the separating chromosomes induce the formation of the contractile ring in the cell's cortex. The ring immediately begins contracting. Contraction continues until it is tight around the midbody and the two halves of the cell are connected by only a thin bridge. Breakage of the bridge separates the two new cells. As with the stages of mitosis, these stages form a continuous process without pauses.

within an anaphase cell that has already begun to form a contractile ring, a second contractile ring will form wherever the spindle comes to rest. The first ring gradually disappears, and as long as the spindle is not moved again the cell will divide at the position of the second. This result is achieved regardless of the distance the spindle is moved from its original position but does not occur if the spindle is moved after a certain point in anaphase. The entire cortex is thus capable of responding to the spindle early in anaphase but later becomes unresponsive.

The spindle itself is positioned within the cell by its astral microtubules. These radiate from both of its poles and are long enough to make extensive contact with much of the cell's cortex, as shown in **FIGURE 10.61**. Spindle positioning in animal cells occurs as the result of interactions between astral microtubules and dynein anchored within the cortex, as **FIGURE 10.62** illustrates. Because dynein moves toward the minus ends of microtubules—which are lo-

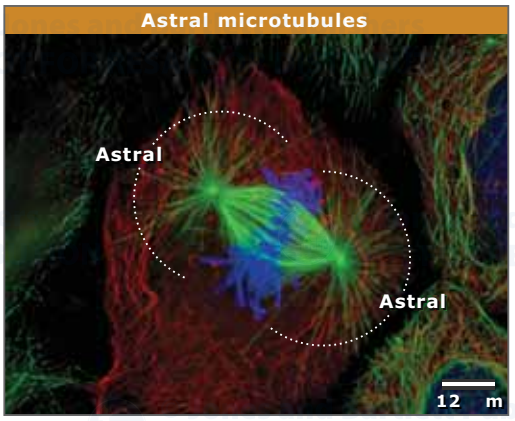

FIGURE 10.61 Astral microtubules radiating from each pole of a metaphase spindle. Microtubules are in green, chromosomes in blue, and intermediate filaments in red. The edge of the intermediate filament array defines the boundary of the cell. Note that many of the astral microtubules extend all the way to the edge of the cell. Photo © Conly Rieder and Alexey Khodjakov, Wadsworth Center.

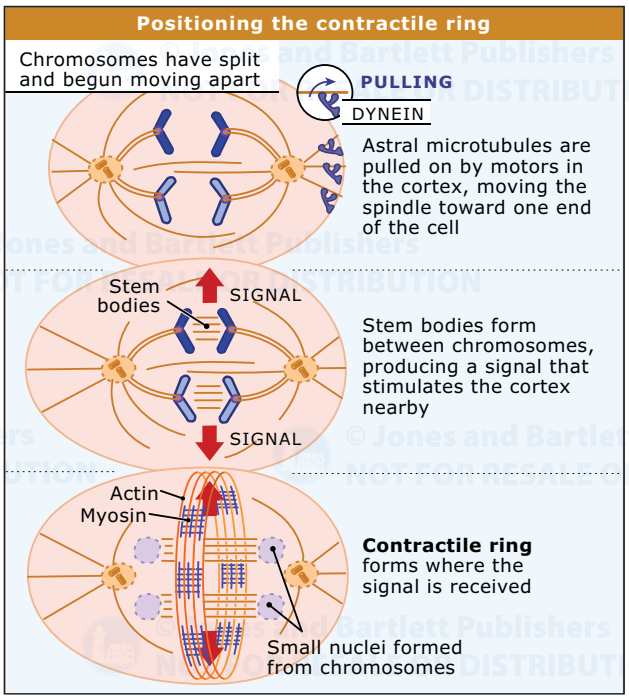

FIGURE 10.62 The position of the contractile ring is determined by a sequence of events. The position of the spindle in the cell is first determined by interactions between its astral microtubules and dynein in the cell cortex. Stem bodies that form as the spindle is being positioned then produce a signal that initiates the assembly of the contractile ring in the nearby cortex.

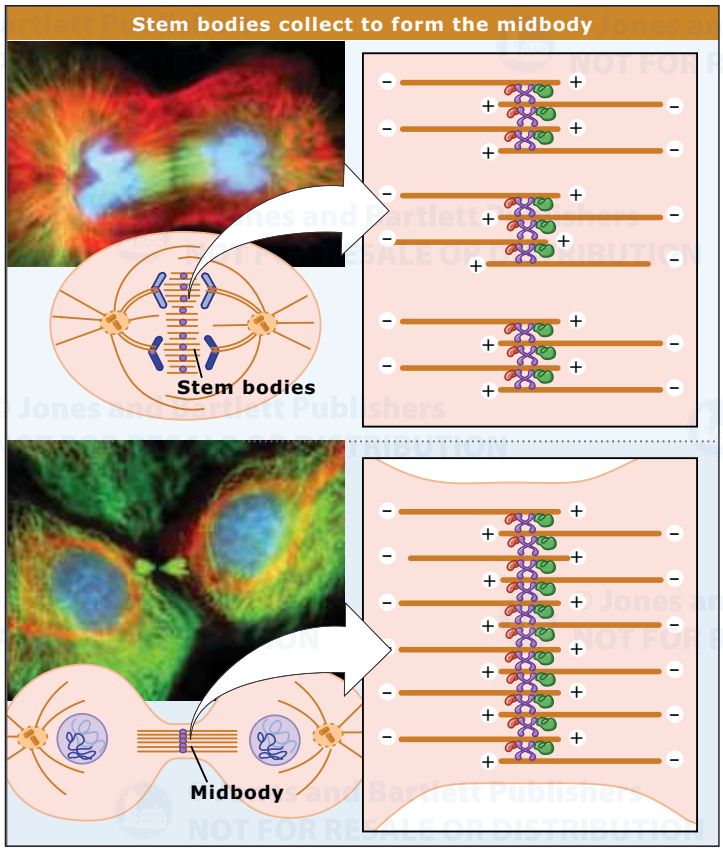

FIGURE 10.63 The upper photograph shows a cell in mid- to late anaphase, its chromosomes (blue) just arriving at the poles. Between the two groups of chromosomes are a broad array of stem bodies (green). The center of each stem body does not stain green because the dense collection of proteins there prevents the stain from binding to the microtubules. The lower photograph shows two sister cells that remain connected only by a midbody. Photos © Conly Rieder, Wadsworth Center.

cated at the poles of the spindle—the result is a pulling force on each of the microtubules. Where dynein is located or activated within the cortex of a cell, the shape of the cell and the relative number of astral microtubules that emanate from the two poles of the spindle determine how the spindle is oriented. It is possible that the spindle's position is determined by a mechanical equilibrium achieved when the pulling forces on the microtubules of its two asters are equal. While there are exceptions, the spindle tends to position itself with its long axis parallel to that of a cell.

In some developing systems and tissues, cytokinesis results in the formation of two daughter cells that differ greatly in size. These asymmetric divisions result from the sudden movement of the spindle during mitosis: in mid-anaphase the spindle quickly shifts its position and moves closer to one side of the cell. As a result of this shift, the cleavage furrow forms off the cell's center. What regulates when the spindle will move and how it moves to a particular position are not yet clear.

How does the spindle determine where the contractile ring forms? Until recently, it was thought that the site of cytokinesis was defined by the spindle's asters. A large number of experiments had led to the conclusion that, as a rule, cytokinesis occurs between two adjacent asters, whether or not they are connected by a mitotic spindle. Factors essential for the contractile ring were proposed to accumulate at the cell membrane where microtubules from the two asters overlap. However, it is now clear that the position of the contractile ring depends not on the asters but instead on another type of microtubule structure that forms between the separating groups of chromosomes early in anaphase.

As the chromosomes separate, many small bundles of microtubules aligned with the spindle form in the region where the chromosomes were aligned at metaphase, as shown in FIGURE 10.63. Called stem bodies or midzone microtubule bundles, these bundles may either be assembled from microtubules shed from the two centrosomes as they separate during anaphase B or formed *de novo* by the polymerization of new microtubules. Each bundle is composed of microtubules of both polarities, with the plus ends of microtubules of opposite polarity overlapping in a small region in the middle of the bundle. These regions of overlap are located midway between the poles of the spindle, so that the bundles are all centered on its midline. The overlap region of each bundle is specialized and contains a unique set of proteins, including microtubule-dependent motor proteins. A kinesin-like motor protein that binds adjacent microtubules of opposite polarity (MKLP1, for *m*itosis *k*inesin-*l*ike *p*rotein 1) is found there and may play a role in the formation of stem bodies by moving to the plus ends of two microtubules simultaneously. Another motor present in the overlap region is CENP-E, which relocates there after having been a component of the kinetochore until the beginning of anaphase, as shown in FIGURE 10.64.

As cytokinesis progresses, the individual stem bodies coalesce in register to form a single large bundle of microtubules, the midbody, that is positioned between the two groups of separated chromosomes (see Figure 10.63 and Figure 10.59). Like each of the individual stem bodies, the central region of the midbody where microtubules of opposite polarity overlap is a specialized structure containing a large number of different types of proteins.

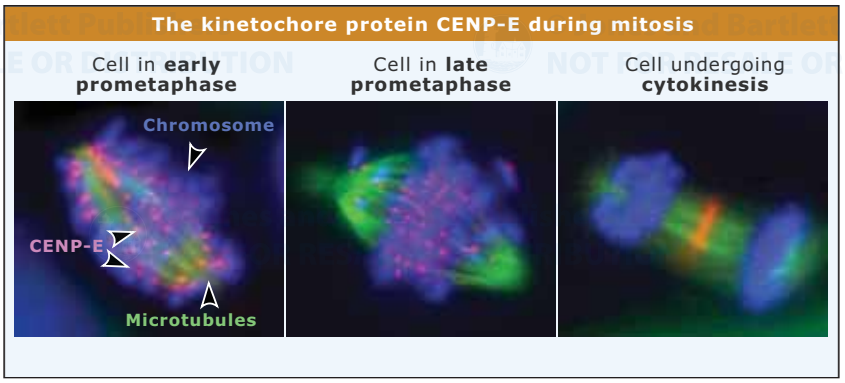

FIGURE 10.64 A series of immunofluorescence micrographs showing how the distibution of CENP-E changes over the course of mitosis. Before and during metaphase it is a component of kinetochores and appears as discrete dots. At the start of anaphase, however, it leaves the kinetochores and accumulates across the equator of the spindle in the narrow zone where the midzone microtubules overlap. CENP-E staining appears purple when it overlaps with DNA and orange when it overlaps with microtubules. Photos courtesy of Bruce F. McEwen, Wadsworth Center.

Stem bodies are required for the formation of the contractile ring and play the important role of determining where it forms. Assembly of the contractile ring involves the organization of a large number of proteins, including its main structural components, actin and myosin. These proteins are initially recruited to a site underneath the plasma membrane where they then assemble into the ring. Both the accumulation and the assembly of the proteins require the presence of stem bodies nearby. In experiments in which stem bodies are prevented from forming, actin and myosin do not accumulate at any site and a contractile ring does not form. Cells can also be experimentally manipulated so that stem bodies are close to the cortex outside the context of a mitotic spindle. When this is done, actin and myosin gather and a contractile ring forms at any point in the cortex that is near a stem body.

Stem bodies, then, appear to be responsible for producing a signal that stimulates the formation of the contractile ring. The molecular mechanism is not yet known, but it is likely that the signal originates within the specialized region at the middle of each stem body where the plus ends of the microtubules are concentrated. Among the proteins localized there is one that catalyzes the activation of the small GTPase Rho. In many other cellular contexts Rho regulates the formation of structures that contain actin, and it is possible that the continuous production and release of active Rho by stem bodies leads to the reorganization of actin and myosin into a contractile ring.

10.22 The contractile ring cleaves the cell in two

Key concepts

- Contraction of the contractile ring causes it to constrict and produces a furrow around the surface of a dividing cell.
- The contractile ring is composed largely of actin and myosin. Its constriction is driven by their interaction.
- Constriction by the contractile ring requires signals from the stem bodies or the midbody.
- A significant amount of membrane fusion is required during cytokinesis.

Once its components have been recruited and assembled at the correct position, the contractile ring begins the task of dividing the cytoplasm in two. Almost as soon as it is forms it begins to contract. As it contracts its diameter decreases steadily until only a small opening is present between the two halves of the cell, each containing one of the two newly formed nuclei (see Figure 10.60 and Figure 10.57). Because the ring is attached to the overlying plasma membrane, its contraction draws the membrane inward between the two nuclei, creating a deep indentation around the surface of the cell, the cleavage furrow. In many unicellular organisms and in cells within the tissues of animals the cleavage furrow is broad and its sides gently sloping, giving a dividing cell the appearance of a dumbbell. In other cells, particularly the large eggs of animals such as sea urchins and frogs, it is a very sharp and deep cleft. In some

cases, a furrow forms on only one side of a cell and divides it by slicing from one side to the other rather than by constriction. These cases reveal that although the contractile apparatus is usually present as a ring, other forms—such as a crescent extending only partway around a cell—can contract as well.

The force for the contraction of the ring is provided by actin and myosin. Like the sarcomeres of muscle, the contractile ring is composed of overlapping filaments of myosin II and actin, and the force for contraction is generated as the two interact and move past one another. Many other proteins are also present in the ring in smaller quantities and serve to organize the actin and myosin or to control their ability to interact and cause contraction. The contractile ring is not simply a small, circular version of a muscle, however. The actin and myosin filaments in the ring are not as precisely arranged as in muscle and are not organized into sarcomeres. The ring is also a much more dynamic structure, quickly disappearing if actin polymerization is blocked with drugs. The dynamic nature of the ring is probably essential for its function, since as it contracts its thickness remains constant. Components must be steadily lost as it decreases in diameter, so much so that as cytokinesis nears completion the great majority of the material that was initially in the ring has been released.

Contraction of the ring is controlled by the microtubules that extend between the separated groups of chromosomes. Ingression of the cleavage furrow requires the continuous presence of stem bodies and may be driven by their coalescence to form the midbody. This is suggested by experiments that demonstrate that the stem bodies collect into a single large bundle even when the contractile ring is prevented from forming by treatment of cells with a drug that prevents actin polymerization.

The signal from the stem bodies to the ring that drives its contraction originates within the special region of microtubule overlap at the middle of each bundle. Ingression requires the continuous activity of several components found there, including a complex of passenger proteins containing the Aurora B kinase. These proteins relocated from the centromere to the overlap region of the stem bodies at the start of anaphase. How they stimulate ingression—whether they are part of the signaling system or are somehow required for the formation of stem bodies—is not yet known. Why these proteins move from one place to another at a critical transition, and what it implies about how the stem bodies are formed and positioned, are intriguing questions that remain to be answered.

Contraction of the ring continues and the cleavage furrow advances until the two halves of a dividing cell are connected by only a thin bridge that contains the midbody (see Figure 10.57 and Figure 10.56). The primary role of this structure is to govern the terminal stage of cytokinesis, when the two daughter cells are finally completely separated. The intercellular bridge can persist for many hours, and cytokinesis is not complete until it finally breaks. This is especially evident when the bridge forms around a piece of chromatin, which sometimes occurs when the chromosomes fail to separate completely during anaphase. When this happens, the bridge fails to break even after many hours, and the furrow finally relaxes to produce a binucleated cell. In normal divisions there appear to be two ways that the bridge can be broken. In some cases it is severed by forces generated as the two daughter cells begin to migrate apart. In others, there exists a specific mechanism in which vesicles delivered to the bridge fuse to close the gap between the two halves of the cell. Different types of cells must use these two mechanisms to different extents. Cells that are not motile, for example, could not rely upon the first mechanism.

In addition to all the cytoskeletal events that take place during cytokinesis a great deal of membrane fusion must also occur. Some fusion events must occur simply to completely separate the two daughter cells. However, membrane fusion events must also occur for a less obvious reason. If a sphere or cube is cleaved in half (which is basically what is happening in cytokinesis), the total volume enclosed by the two halves produced is the same as that of the original object. Their total surface area, however, is much greater since the cleavage produces new surfaces, as **FIGURE 10.65** illustrates. This means that a significant amount of new plasma membrane must be added as cytokinesis occurs. This process has been studied in detail in animal cells. There, the expansion of surface area occurs by the fusion of internal vesicles with the plasma membrane immediately behind the leading edge of the furrow. This process is particularly clear in the eggs of some amphibians, such as frogs. These eggs are extremely large and must undergo many successive divisions as rapidly as possible. Because this strategy does not allow time for the synthesis of large amounts of new material during each cell cycle, the cytoplasm

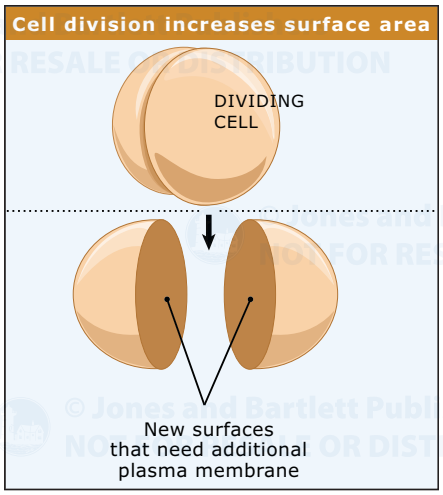

FIGURE 10.65 The combined surface area of the two daughter cells that result from cell division is greater than the surface area of the mother cell. As a result, a significant amount of new plasma membrane must be added during cytokinesis.

of each egg contains a large stockpile of vesicles waiting to be added to the plasma membrane each time the cell divides.

10.23 The segregation of nonnuclear organelles during cytokinesis is based on chance

Key concept

- Many of the cell's internal membranes break down during mitosis and are distributed between the two daughter cells as small vesicles. These vesicles re-form the organelle after mitosis is finished.

The division of a cell entails more than just separation of the replicated centrosomes and chromosomes into two daughter cells. Each new daughter must also inherit enough of the original cytoplasm and organelles to ensure its viability. For many organelles *this distribution is accomplished by first breaking them down into a large number of small subunits and then dispersing these subunits randomly throughout the volume of the mother cell.* In general, disassembly and dispersion occur in response to the activation of the master mitotic regulatory kinase, cyclin B/CDK1.

Almost all of a cell's structural components and membrane-bound organelles are affected. During the mitotic state the components of the endomembrane system, including the Golgi apparatus, the rough and smooth endoplasmic reticula, and the nuclear envelope, all fragment. The fragments generated then become randomly distributed throughout the cell, often by associating with the highly dynamic microtubules growing from the two asters. With the exception of the microtubules (which become remodeled to form the spindle) the components of the cytoskeletal system also undergo significant disassembly. The subunits released from the disassembly of actin and intermediate filaments also diffuse throughout the cell. In most cases, the breakdown of the cytoskeleton induces the cell to round up into a sphere. This radical morphological change during the early stages of mitosis undoubtedly helps disperse the components throughout the volume of the cell.

About the only cytoplasmic organelles that do not disassemble or fragment as mitosis begins are mitochondria. Mitochondria appear unaffected at the structural level during mitosis and probably do not break down because they are already present in multiple small copies. Individual mitochondria function throughout the cell cycle as independent units, and there are hundreds or thousands of them per cell. In essence, the function of ATP generation is already extensively fragmented and dispersed. In contrast, the Golgi apparatus is present in only a single copy in animal cells, requiring that it be broken down in order to distribute its function between the two daughter cells.

As a result of all the disassembly events that take place early in mitosis, by the time a cell enters anaphase most of its components are present in multiple copies that are distributed randomly throughout its volume. After cytokinesis occurs and the cytoplasm is divided into two roughly equivalent volumes, the two cells each end up with a centrosome, a single complement of chromosomes, and roughly similar quantities of precursors for their organellar and cytoskeletal systems. Then, as cyclin B/CDK1 is inactivated and the cell exits the mitotic state, the processes that led to the breakdown of its internal systems are reversed and they reform.

10.24 What's next?

The quest to understand how mitosis works is not simply an academic challenge. The genetic changes associated with the origins of many diseases can often be traced directly to mistakes that occurred during the division process. With modern genetic and molecular tools, it

is now relatively easy to discover new molecules that are involved in chromosome segregation and cytokinesis. The key will be to determine the function of these molecules, how they work together to accomplish a particular event, and then how the multiple events of mitosis are integrated to effect one of the most fundamental processes of life.

We still have much to learn about how the individual proteins that participate in mitosis work during the process. The development of increasingly sophisticated imaging systems, as well as methods for fluorescently tagging specific proteins in the living cell, will provide increasingly accurate views of how the components of the spindle interact. Coupled with broadly applicable methods that allow the inactivation or removal of a specific protein from cells—such as RNAi—these techniques should produce a much clearer picture of how each player affects entry into and/or progression through mitosis. No doubt, one area of emphasis will be to define the molecular mechanism behind centrosome replication, how this replication is coordinated with DNA synthesis, and how the cell controls the number of copies of this important organelle. It is now clear that early in the development of many cancerous cells centrosomes are overproduced. Extra centrosomes lead to the formation of multipolar spindles and the production of aneuploid cells. In the future it will be important to determine how this overproduction of centrosomes occurs and whether it has a causative role in the early stages of the formation of tumors.

Another exciting area for future investigation will be defining how the spindle assembly checkpoint works at the molecular level. How can the signal produced by just one kinetochore inhibit anaphase onset throughout the cell? Malfunctions in this checkpoint also lead directly to aneuploidy and its disastrous consequences, including tumor formation. Many cancer cells do not contain a workable spindle assembly checkpoint. Thus, understanding how the wait-anaphase signal inhibits the anaphase-promoting complex will assist in the development of drugs to kill selected dividing cells.

No doubt another important area for future work will be to determine how microtubule subunit flux occurs and the role it plays in chromosome positioning and motion. Is flux due to motor proteins anchored within the spindle matrix that act along the length of the microtubules? Is it due to the action of specific proteins only at the ends of the microtubules? Is the pole-directed force on the kinetochore produced by microtubule subunit flux much greater than that produced by microtubule motors associated with the kinetochore? And what are all the events happening at the kinetochore and how are they coordinated with one another? The answer to these questions will provide important insight into how chromosomes become positioned on the equator of the spindle.

Finally, we have an incomplete understanding of how the cell controls entry into mitosis. It is clear that cells will not enter mitosis when their DNA is damaged. However, it is increasingly clear that many variables in addition to the integrity of the genome control entry into mitosis. For each the cell appears to have a checkpoint pathway. Some of these monitor the function of microtubules. Others are triggered by various drugs and environmental stresses. Understanding these pathways and how they stop the cell from entering mitosis will help lead to great advances in the prevention and cure of many devastating diseases.

10.25 Summary

The process of cell division is an intensively investigated area of biomedical research because errors in mitosis lead to aneuploidy and the genetic instability behind cancer. Mitosis occurs through two processes, nuclear division and cytokinesis. Nuclear division, or karyokinesis, equally distributes the replicated sister chromatids into two daughter nuclei. Near the end of nuclear division, cytokinesis partitions the cell and its cytoplasm between the two daughter nuclei.

The mitotic apparatus, or spindle, mediates nuclear division and cytokinesis by moving the chromosomes and defining the plane that bisects the cell. This bipolar structure is composed primarily of microtubules, microtubule associated proteins (including motors), and structural proteins. The microtubules within the mature spindle are of two types: those that firmly connect the sister kinetochores on each chromosome to the opposing spindle poles, and those that have free ends within the spindle. The dynamic nature of microtubules is critical to spindle formation and function.

Two distinct mechanisms underlie the motion of kinetochores, and thus their associated

chromosomes, toward a spindle pole. One force is generated by shortening of the kinetochore-associated microtubules, which occurs through subunit deletion in the spindle pole region. The other involves microtubule motors associated with the kinetochore. In some cells, like those of vertebrates, these mechanisms work concurrently.

A complex cell cycle checkpoint control delays the metaphase/anaphase transition until all of the kinetochores are stably attached to the spindle. When the checkpoint is turned off, a series of biochemical changes leads to the disjunction of sister chromatids, the dissolution of the spindle proper, and the formation of stem bodies between the separating groups of chromosomes. The stem bodies then initiate the process of cytokinesis.

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