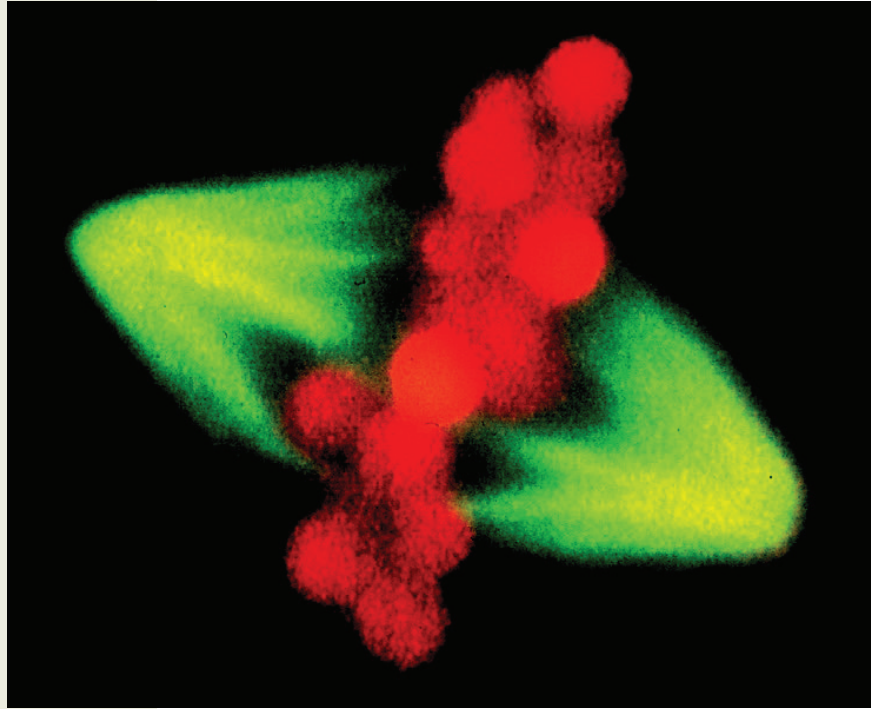


14



Cellular Reproduction

14.1 The Cell Cycle

14.2 M Phase: Mitosis and Cytokinesis

14.3 Meiosis

THE HUMAN PERSPECTIVE: Meiotic Nondisjunction and Its Consequences

EXPERIMENTAL PATHWAYS: The Discovery and Characterization of MPF

According to the third tenet of the cell theory, new cells originate only from other living cells. The process by which this occurs is called **cell division**. For a multicellular organism, such as a human or an oak tree, countless divisions of a single-celled zygote produce an organism of astonishing cellular complexity and organization. Cell division does not stop with the formation of the mature organism but continues in certain tissues throughout life. Millions of cells residing within the marrow of your bones or the lining of your intestinal tract are undergoing division at this very moment. This enormous output of cells is needed to replace cells that have aged or died.

Although cell division occurs in all organisms, it takes place very differently in prokaryotes and eukaryotes. We will restrict discussion to the eukaryotic version. Two distinct types of eukaryotic cell division will be discussed in this chapter. Mitosis leads to production of cells that are genetically identical to their parent, whereas meiosis leads to production of cells with half the genetic content of the parent. Mitosis serves as the basis for producing new cells, meiosis as the basis for producing new

Fluorescence micrograph of a mitotic spindle that had assembled in a cell-free extract prepared from frog eggs, which are cells that lack a centrosome. The red spheres consist of chromatin-covered beads that were added to the extract. It is evident from this micrograph that a bipolar spindle can assemble in the absence of both chromosomes and centrosomes. In this experiment, the chromatin-covered beads served as nucleating sites for the assembly of the microtubules that subsequently formed this spindle. The mechanism by which cells construct mitotic spindles in the absence of centrosomes is discussed on page 587. (FROM R. HEALD, ET AL., NATURE VOL. 382, COVER OF 8/1/96; © 1996, REPRINTED WITH PERMISSION FROM MACMILLAN PUBLISHERS, LIMITED.)

sexually reproducing organisms. Together, these two types of cell division form the links in the chain between parents and their

offspring and, in a broader sense, between living species and the earliest eukaryotic life forms present on Earth.

14.1 | The Cell Cycle



In a population of dividing cells, whether inside the body or in a culture dish, each cell passes through a series of defined stages, which constitutes the **cell cycle** (Figure 14.1). The cell cycle can be divided into two major phases based on cellular activities readily visible with a light microscope: **M phase** and **interphase**. **M phase** includes (1) the process of **mitosis**, during which duplicated chromosomes are separated into two nuclei, and (2) **cytokinesis**, during which the entire cell divides into two daughter cells. **Interphase**, the period between cell divisions, is a time when the cell grows and engages in diverse metabolic activities. Whereas M phase usually lasts only an hour or so in mammalian cells, interphase may extend for days, weeks, or longer, depending on the cell type and the conditions.

Although M phase is the period when the contents of a cell are actually divided, numerous preparations for an upcoming mitosis occur during interphase, including replication of the cell's DNA. One might guess that a cell engages in replication throughout interphase. However, studies in the early 1950s on asynchronous cultures (i.e., cultures whose cells are randomly distributed throughout the cell cycle) showed that this is not the case. As described in Chapter 13, DNA replication can be monitored by the incorporation of [^3H]thymidine into newly syn-

thesized DNA. If [^3H]thymidine is given to a culture of cells for a short period (e.g., 30 minutes) and a sample of the cell population is fixed, dried onto a slide, and examined by autoradiography, only a fraction of the cells are found to have radioactive nuclei. Among cells that were engaged in mitosis at the time of fixation (as evidenced by their compacted chromosomes) none is found to have a radioactively labeled nucleus. These mitotic cells have unlabeled chromosomes because they were not engaged in DNA replication during the labeling period.

If labeling is allowed to continue for one or two hours before the cells are sampled, there are still no cells with labeled mitotic chromosomes (Figure 14.2). We can conclude from these results that there is a definite period of time between the end of DNA synthesis and the beginning of M phase. This period is termed G_2 (for second gap). The duration of G_2 is revealed as one continues to take samples of cells from the culture until labeled mitotic chromosomes are observed. The first cells whose mitotic chromosomes are labeled must have been at the last stages of DNA synthesis at the start of the incubation with [^3H]thymidine. The length of time between the start of the labeling period and the appearance of cells with labeled mitotic figures corresponds to the duration of G_2 .

DNA replication occurs during a period of the cell cycle termed **S phase**. S phase is also the period when the cell synthesizes the additional histones that will be needed as the cell doubles the number of nucleosomes in its chromosomes (see Figure 13.23). The length of S phase can be determined directly. In an asynchronous culture, the percentage of cells engaged in a particular activity is an approximate measure of the percentage of time that this activity occupies in the lives of cells. Thus, if we know the length of the entire cell cycle, the

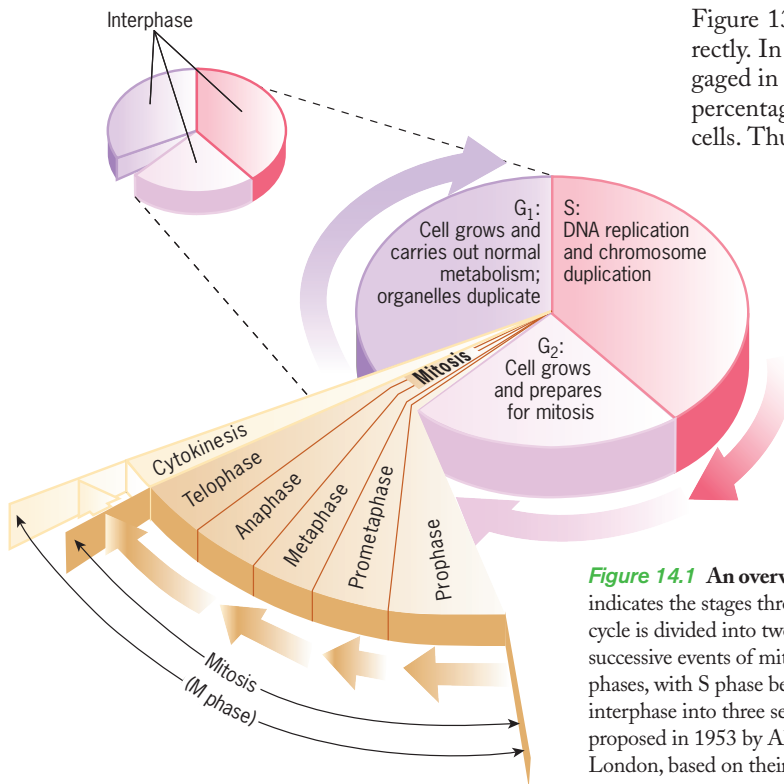


Figure 14.1 An overview of the eukaryotic cell cycle. This diagram of the cell cycle indicates the stages through which a cell passes from one division to the next. The cell cycle is divided into two major phases: M phase and interphase. M phase includes the successive events of mitosis and cytokinesis. Interphase is divided into G_1 , S, and G_2 phases, with S phase being equivalent to the period of DNA synthesis. The division of interphase into three separate phases based on the timing of DNA synthesis was first proposed in 1953 by Alma Howard and Stephen Pelc of Hammersmith Hospital, London, based on their experiments on plant meristem cells.

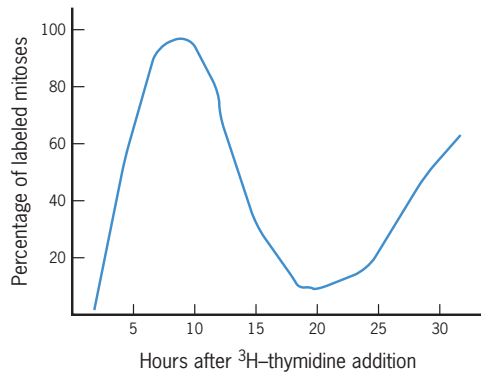


Figure 14.2 Experimental results demonstrating that replication occurs during a defined period of the cell cycle. HeLa cells were cultured for 30 minutes in medium containing [³H]thymidine and then incubated (chased) for various times in unlabeled medium before being fixed and prepared for autoradiography. Each culture dish was scanned for cells that were in mitosis at the time they were fixed, and the percentage of those mitotic cells whose chromosomes were labeled was plotted as shown. (FROM A STUDY BY R. BASERGA AND F. WIEBEL.)

length of S phase can be calculated directly from the percentage of the cells whose nuclei are radioactively labeled during a brief pulse with [³H]thymidine. Similarly, the length of M phase can be calculated from the percentage of cells in the population that are seen to be engaged in mitosis or cytokinesis. When one adds up the periods of G₂ + S + M, it is apparent that there is an additional period in the cell cycle yet to be accounted for. This other phase, termed G₁ (for first gap), is the period following mitosis and preceding DNA synthesis.

Cell Cycles in Vivo

One of the properties that distinguishes various types of cells within a multicellular plant or animal is their capacity to grow and divide. We can recognize three broad categories of cells:

1. **Cells, such as nerve cells, muscle cells, or red blood cells, that are highly specialized and lack the ability to divide.** Once these cells have differentiated, they remain in that state until they die.
2. **Cells that normally do not divide but can be induced to begin DNA synthesis and divide when given an appropriate stimulus.** Included in this group are liver cells, which can be induced to proliferate by the surgical removal of part of the liver, and lymphocytes, which can be induced to proliferate by interaction with an appropriate antigen.
3. **Cells that normally possess a relatively high level of mitotic activity.** Included in this category are stem cells of various adult tissues, such as hematopoietic stem cells that give rise to red and white blood cells (Figure 17.6) and stem cells at the base of numerous epithelia that line the body cavities and the body surface (Figure 7.1). The relatively unspecialized cells of apical meristems located near the tips of plant roots and stems also exhibit rapid and continual cell division. Stem cells have an important property that is not shared by most cells; they are able to divide asymmetrically.

An **asymmetric cell division** is one in which the two daughter cells have different sizes, components, or fates. The asymmetric division of a stem cell produces one daughter cell that remains an uncommitted stem cell like its parent and another daughter cell that has taken a step towards becoming a differentiated cell of that tissue. In other words, asymmetric divisions allow stem cells to engage in both self-renewal and the formation of differentiated tissue cells. Some types of nonstem cells can also engage in asymmetric (or unequal) cell divisions, as illustrated by the formation of oocytes and polar bodies in Figure 14.41*b* and the division of the T cell in the Chapter 17 opening photo.

Cell cycles can range in length from as short as 30 minutes in a cleaving frog embryo, whose cell cycles lack both G₁ and G₂ phases, to several months in slowly growing tissues, such as the mammalian liver. Many cells in the body are said to be *quiescent*, which means that they are in a state that will not lead them to an upcoming cell division, but they retain the capability to divide if conditions should change. With a few notable exceptions, cells that have stopped dividing are arrested in a stage preceding the initiation of DNA synthesis. Quiescent cells are often described as being in the G₀ state to distinguish them from the typical G₁-phase cells that may soon enter S phase. A cell must receive a growth-promoting signal to proceed from G₀ into G₁ phase and thus reenter the cell cycle.

Control of the Cell Cycle

The study of the cell cycle is not only important in basic cell biology, but also has enormous practical implications in combating cancer, a disease that results from a breakdown in a cell's ability to regulate its own division. In 1970, a series of cell fusion experiments carried out by Potu Rao and Robert Johnson of the University of Colorado helped open the door to understanding how the cell cycle is regulated.

Rao and Johnson wanted to know whether the cytoplasm of cells contains regulatory factors that affect cell cycle activities. They approached the question by fusing mammalian cells that were in different stages of the cell cycle. In one experiment, they fused mitotic cells with cells in other stages of the cell cycle. The mitotic cell always induced compaction of the chromatin in the nucleus of the nonmitotic cell (Figure 14.3). If a G₁-phase and an M-phase cell were fused, the chromatin of the G₁-phase nucleus underwent *premature chromosomal compaction* to form a set of elongated compacted chromosomes (Figure 14.3*a*). If a G₂-phase and M-phase cell were fused, the G₂ chromosomes also underwent premature chromosome compaction, but unlike those of a G₁ nucleus, the compacted G₂ chromosomes were visibly doubled, reflecting the fact that replication had already occurred (Figure 14.3*c*). If a mitotic cell was fused with an S-phase cell, the S-phase chromatin also became compacted (Figure 14.3*b*). However, replicating DNA is especially sensitive to damage, so that compaction in the S-phase nucleus led to the formation of "pulverized" chromosomal fragments rather than intact, compacted chromosomes. The results of these experiments suggested that the cytoplasm of a mitotic cell contained diffusible factors that could induce mitosis in a nonmitotic (i.e., inter-

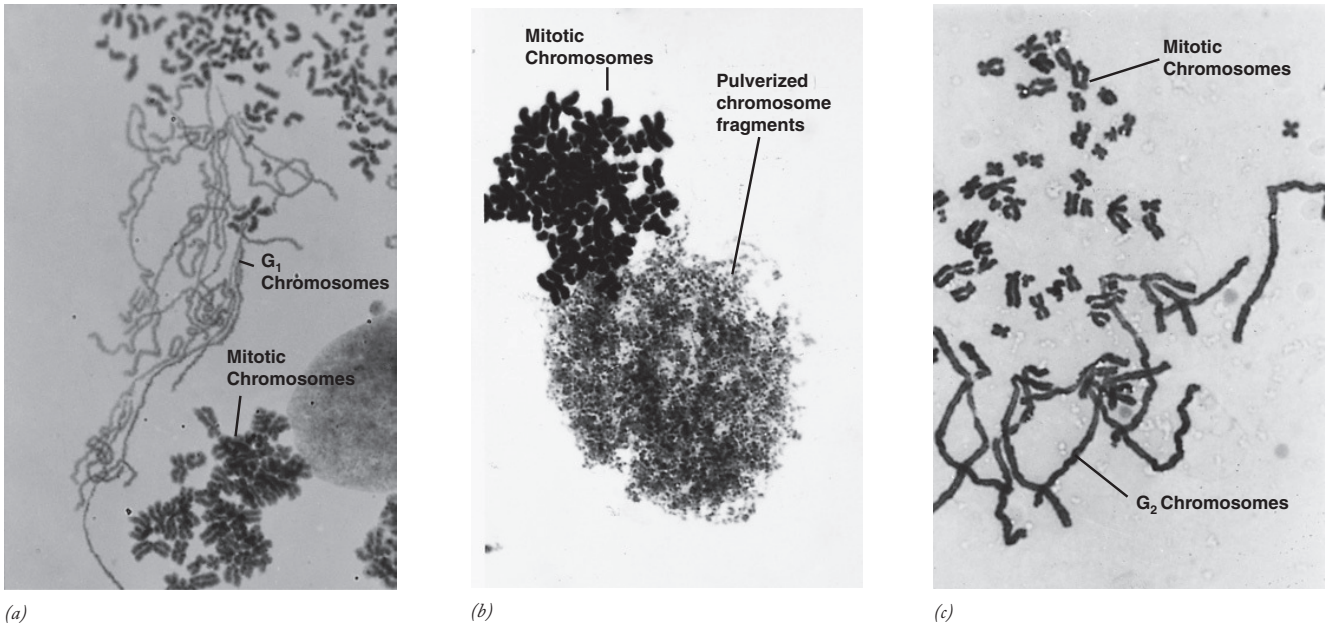


Figure 14.3 Experimental demonstration that cells contain factors that stimulate entry into mitosis. The photographs show the results of the fusion of an M-phase HeLa cell with a rat kangaroo PtK2 cell that had been in (a) G₁ phase, (b) S phase, or (c) G₂ phase at the time of cell fusion. As described in the text, the chromatin of the G₁-phase and G₂-phase PtK2 cells undergoes premature compaction, whereas that of

the S-phase cell becomes pulverized. The elongated chromatids of the G₂-phase cell in *c* are doubled in comparison with those of the G₁ cell in *a*. (FROM KARL SPERLING AND POTU N. RAO, *HUMAN GENETICS* 23:437, 1974. WITH KIND PERMISSION OF SPRINGER SCIENCE+ BUSINESS MEDIA.)

phase) cell. This finding suggested that the transition from G₂ to M was under positive control; that is, the transition was induced by the presence of some stimulatory agent.

The Role of Protein Kinases While the cell-fusion experiments revealed the existence of factors that regulated the cell cycle, they provided no information about the biochemical properties of these factors. Insights into the nature of the agents that promote entry of a cell into mitosis (or meiosis) were first gained in a series of experiments on the oocytes and early embryos of frogs and invertebrates. These experiments are described in the Experimental Pathways at the end of this chapter. To summarize here, it was shown that entry of a cell into M phase is initiated by a protein called *maturation-promoting factor* (MPF). MPF consists of two subunits: (1) a subunit with kinase activity that transfers phosphate groups from ATP to specific serine and threonine residues of specific protein substrates and (2) a regulatory subunit called *cyclin*. The term *cyclin* was coined because the concentration of this regulatory protein rises and falls in a predictable pattern with each cell cycle (Figure 14.4). When the cyclin concentration is low, the kinase lacks the cyclin subunit and, as a result, is inactive. When the cyclin concentration rises, the kinase is activated, causing the cell to enter M phase. These results suggested that (1) progression of cells into mitosis depends on an enzyme whose sole activity is to phosphorylate other proteins, and (2) the activity of this enzyme is controlled by a subunit whose concentration varies from one stage of the cell cycle to another.

Over the past two decades, a large number of laboratories have focused on MPF-like enzymes, called **cyclin-dependent**

kinases (Cdks). It has been found that Cdks are not only involved in M phase but are the key agents that orchestrate activities throughout the cell cycle. Cdks carry out this function

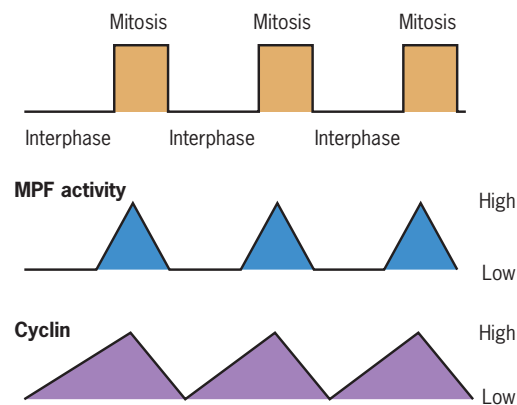


Figure 14.4 Fluctuation of cyclin and MPF levels during the cell cycle. This drawing depicts the cyclical changes that occur during early frog development when mitotic divisions occur rapidly and synchronously in all cells of the embryo. The top tracing shows the alternation between periods of mitosis and interphase, the middle tracing shows the cyclical changes in MPF kinase activity, and the lower tracing shows the cyclical changes in the concentrations of cyclins that control the relative activity of the MPF kinase. (FROM A. W. MURRAY AND M. W. KIRSCHNER, *SCIENCE* 246:616, 1989; COPYRIGHT 1989, AAAS. SCIENCE BY MOSES KING, REPRODUCED WITH PERMISSION OF AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE IN THE FORMAT REUSE IN A BOOK/TEXTBOOK VIA COPYRIGHT CLEARANCE CENTER.)

by phosphorylating a diverse array of proteins. Each phosphorylation event occurs at an appropriate point during the cell cycle, thereby stimulating or inhibiting a particular cellular process involved in cell division. Yeast cells have been particularly useful in studies of the cell cycle, at least in part because of the availability of temperature-sensitive mutants whose abnormal proteins affect various cell cycle processes. As discussed on page 549, temperature-sensitive mutants can be grown in a relatively normal manner at a lower (permissive) temperature and then shifted to a higher (restrictive) temperature to study the effect of the mutant gene product.

Researchers studying the genetic control of the cell cycle have focused on two distantly related yeast species, the budding yeast *Saccharomyces cerevisiae*, which reproduces through the formation of buds at one end of the cell (see Figure 1.18*b*), and the fission yeast, *Schizosaccharomyces pombe*, which reproduces by elongating itself and then splitting into two equal-sized cells (see Figure 14.6). The molecular basis of cell cycle regulation has been remarkably conserved throughout the evolution of eukaryotes. Once a gene involved in cell cycle control has been identified in one of the two yeast species, homologues are sought—and usually found—in the genomes of higher eukaryotes, including humans. By combining genetic, biochemical, and live-cell analyses, investigators have gained a comprehensive understanding of the major activities that allow a cell to grow and reproduce in a laboratory culture dish.

Research into the genetic control of the cell cycle in yeast began in the 1970s in two laboratories, initially that of Leland Hartwell at the University of Washington working on budding yeast and subsequently that of Paul Nurse at the University of Oxford working on fission yeast. Both laboratories identified a gene that, when mutated, would cause the growth of cells at elevated temperature to stop at certain points in the cell cycle. The product of this gene, which was called *cdc2* in fission yeast (and *CDC28* in budding yeast), was eventually found to be homologous to the catalytic subunit of MPF; in other words, it was a cyclin-dependent kinase. Subsequent research on yeast as well as many different vertebrate cells has supported the concept that the progression of a eukaryotic cell through its cell cycle is regulated at distinct stages. One of the primary stages of regulation occurs near the end of G_1 and another near the end of G_2 . These stages represent points in the cell cycle where a cell becomes committed to beginning a crucial event—either initiating replication or entering mitosis.

We will begin our discussion with fission yeast, which has the least complex cell cycle. In this species, the same Cdk (*cdc2*) is responsible for passage through both points of commitment, though in partnership with different cyclins. A simplified representation of cell cycle regulation in fission yeast is shown in Figure 14.5. The first transition point, which is called START, occurs in late G_1 . Once a cell has passed START, it is irrevocably committed to replicating its DNA and, ultimately, completing the cell cycle.¹ Passage through

¹Mammalian cells pass through a comparable point during G_1 , referred to as the *restriction point*, at which time they become committed to DNA replication and ultimately to completing mitosis. Prior to the restriction point, mammalian cells require the presence of growth factors in their culture medium if they are to progress in the cell cycle. After they have passed the restriction point, these same cells will continue through the remainder of the cell cycle without external stimulation.

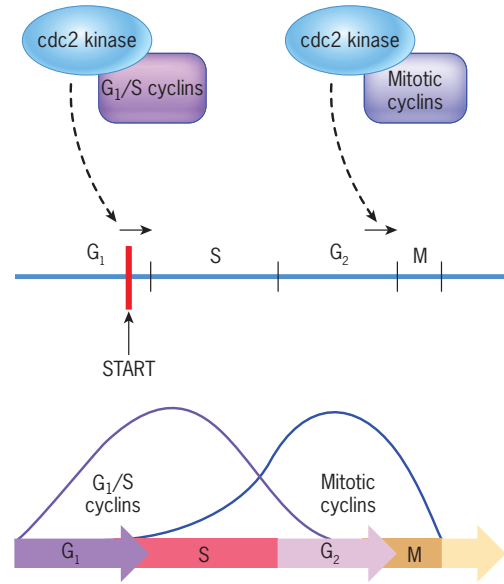


Figure 14.5 A simplified model for cell cycle regulation in fission yeast. The cell cycle is controlled primarily at two points, START and the G_2 –M transition. Passage of a cell through these two critical junctures (black arrows) requires the activation of the same *cdc2* kinase by different classes of cyclins, either G_1 /S or mitotic cyclins. A third major transition occurs at the end of mitosis and is triggered by a rapid drop in concentration of mitotic cyclins. (Note: *cdc2* is also known as Cdk1.)

START requires the activation of *cdc2* by one or more G_1 /S cyclins, whose levels rise during late G_1 (Figure 14.5).

Passage from G_2 to mitosis requires activation of *cdc2* by a different group of cyclins—the *mitotic cyclins*. Cdks containing a mitotic cyclin (e.g., MPF described on page 611) phosphorylate substrates that are required for the cell to enter mitosis. Included among the substrates are proteins required for the dynamic changes in organization of both the chromosomes and cytoskeleton that characterize the shift from interphase to mitosis. Cells make a third commitment during the middle of mitosis, which determines whether they will complete cell division and reenter G_1 of the next cycle. Exit from mitosis and entry into G_1 depends on a rapid decrease in Cdk activity that results from a plunge in concentration of the mitotic cyclins (Figure 14.5), an event that will be discussed on page 592 in conjunction with other mitotic activities.

Cyclin-dependent kinases are often described as the “engines” that drive the cell cycle through its various stages. The activities of these enzymes are regulated by a variety of “brakes” and “accelerators” that operate in combination with one another. These include:

Cyclin Binding As indicated in Figure 14.5, the levels of particular cyclins rise over time. When a cyclin reaches a sufficient concentration in the cell, it binds to the catalytic subunit of a Cdk, causing a major change in the conformation of the enzyme’s active site. X-ray crystallographic structures of various cyclin–Cdk complexes indicate that cyclin binding causes

the movement of a flexible loop of the Cdk polypeptide chain away from the opening of the active site, allowing the Cdk to phosphorylate its protein substrates.

Cdk Phosphorylation/dephosphorylation We have already seen in other chapters that many events that take place in a cell are regulated by the addition and removal of phosphate groups from proteins. The same is true for the events that lead to the onset of mitosis. We can see from Figure 14.5 that the level of mitotic cyclins rises through S and G₂. The mitotic cyclins present in a yeast cell during this period bind to the Cdk to form a cyclin–Cdk complex, but the complex shows little evidence of kinase activity. Then, late in G₂, the cyclin–Cdk becomes activated and mitosis is triggered. To understand this change in Cdk activity, we have to look at the activity of three other regulatory enzymes—two kinases and a phosphatase. We will look briefly at the events that occur in fission yeast (Figure 14.6a). The roles of these enzymes in the fission yeast cycle, which is illustrated in Figure 14.6b, was revealed through a combination of genetic and biochemical analyses. In step 1, one of the kinases, called CAK (Cdk-activating kinase), phosphorylates a critical threonine residue (Thr 161 of *cdc2* in Figure 14.6b). Phosphorylation of this residue is necessary, but not sufficient, for the Cdk to be active. A second protein kinase shown in step 1, called Wee1, phosphorylates a key tyrosine residue in the ATP-binding pocket of the enzyme (Tyr 15 of *cdc2* in Figure 14.6b). If this residue is phosphorylated, the enzyme is inactive, regardless of the phosphorylation state of any

other residue. In other words, the effect of Wee1 overrides the effect of CAK, keeping the Cdk in an inactive state. Line 2 of Figure 14.6c shows the phenotype of cells with a mutant *wee1* gene. These mutants cannot maintain the Cdk in an inactive state and divide at an early stage in the cell cycle producing smaller cells, hence the name “wee.” In normal (wild-type) cells, Wee1 keeps the Cdk inactive until the end of G₂. Then, at the end of G₂, the inhibitory phosphate at Tyr 15 is removed by the third enzyme, a phosphatase named Cdc25 (step 2, Figure 14.6b). Removal of this phosphate switches the stored cyclin–Cdk molecules into the active state, allowing it to phosphorylate key substrates and drive the yeast cell into mitosis. Line 3 of Figure 14.6c shows the phenotype of cells with a mutant *cdc25* gene. These mutants cannot remove the inhibitory phosphate from the Cdk and cannot enter mitosis. The balance between Wee1 kinase and Cdc25 phosphatase activities, which normally determines whether the cell will remain in G₂ or progress into mitosis, is regulated by still other kinases and phosphatases. As we will see shortly, these pathways can stop the cell from entering mitosis under conditions that might lead to an abnormal cell division.

Cdk Inhibitors Cdk activity can be blocked by a variety of inhibitors. In budding yeast, for example, a protein called Sic1 acts as a Cdk inhibitor during G₁. The degradation of Sic1 allows the cyclin–Cdk that is present in the cell to initiate DNA replication. The role of Cdk inhibitors in mammalian cells is discussed on page 581.

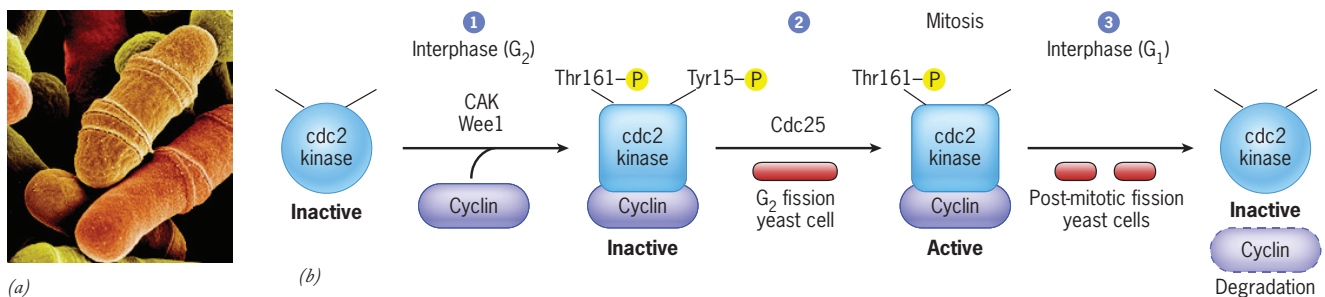
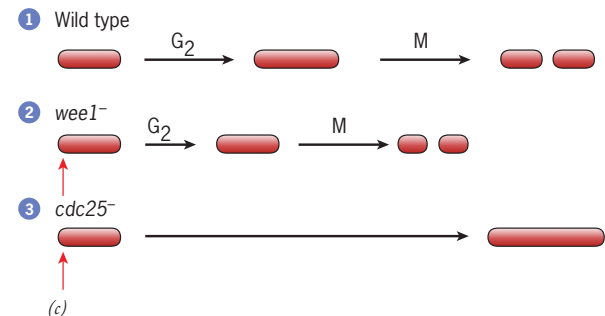


Figure 14.6 Progression through the fission yeast cell cycle requires the phosphorylation and dephosphorylation of critical *cdc2* residues. (a) Colorized scanning electron micrograph of wild type fission yeast cells. (b) During G₂, the *cdc2* kinase interacts with a mitotic cyclin but remains inactive as the result of phosphorylation of a key tyrosine residue (Tyr 15 in fission yeast) by Wee1 (step 1). A separate kinase, called CAK, transfers a phosphate to another residue (Thr 161), which is required for *cdc2* kinase activity later in the cell cycle. When the cell reaches a critical size, an enzyme called Cdc25 phosphatase is activated, which removes the inhibitory phosphate on the Tyr 15 residue. The resulting activation of the *cdc2* kinase drives the cell into mitosis (step 2). By the end of mitosis (step 3), the stimulatory phosphate group is removed from Thr 161 by another phosphatase. The free cyclin is subsequently degraded, and the cell begins another cycle. (The mitotic Cdk in mammalian cells is phosphorylated and dephosphorylated in a similar manner.) (c) Identification of Wee1 kinase and Cdc25 phosphatase was made by studying mutants that behaved as shown in this figure. Line 1 shows the G₂ and M stages of a wild-type cell. Line 2 shows the effect of a mutant *wee1* gene; the cell divides prematurely, forming small (wee) cells. Line 3 shows the effect of a



mutant *cdc25* gene; the cell does not divide but continues to grow. The red arrow marks the time when the temperature is raised to inactivate the mutant protein. (A: STEVE GSCHMEISSNER/PHOTO RESEARCHERS, INC. B: AFTER T. R. COLEMAN AND W. G. DUNPHY, CURR. OPIN. CELL BIOL. 76:877, 1994. CURRENT OPINION IN CELL BIOLOGY BY ELSEVIER LTD. REPRODUCED WITH PERMISSION OF ELSEVIER LTD. IN THE FORMAT REUSE IN A BOOK/TEXTBOOK VIA COPYRIGHT CLEARANCE CENTER.)

Controlled Proteolysis It is evident from Figures 14.4 and 14.5 that cyclin concentrations oscillate during each cell cycle, which leads to changes in the activity of Cdks. Cells regulate the concentration of cyclins, and other key cell cycle proteins, by adjusting both the rate of synthesis and the rate of destruction of the molecule at different points in the cell cycle. Degradation is accomplished by means of the ubiquitin–proteasome pathway described on page 541. Unlike other mechanisms that control Cdk activity, degradation is an irreversible event that helps drive the cell cycle in a single direction. Regulation of the cell cycle requires two classes of multisubunit complexes (SCF and APC complexes) that function as *ubiquitin ligases*. These complexes recognize proteins to be degraded and link these proteins to a polyubiquitin chain, which ensures their destruction in a proteasome. The SCF complex is active from late G_1 through early mitosis (see Figure 14.26a) and mediates the destruction of G_1/S cyclins, Cdk inhibitors, and other cell cycle proteins. These proteins become targets for an SCF after they are phosphorylated by the protein kinases (i.e., Cdks) that regulate the cell cycle. Mutations that inhibit SCFs from mediating proteolysis of key proteins, such as G_1/S cyclins or the Cdk inhibitor Sic1 mentioned above, can prevent cells from entering S phase and replicating their DNA. The APC complex acts in mitosis and degrades a number of key mitotic proteins, including the mitotic cyclins. Destruction of the mitotic cyclins allows a cell to exit mitosis and enter a new cell cycle (page 592).

Subcellular Localization Cells contain a number of different compartments in which regulatory molecules can either be united with or separated from the proteins they interact with. Subcellular localization is a dynamic phenomenon in which cell cycle regulators are moved into different compartments at different stages. For example, one of the major mitotic cyclins in animal cells (cyclin B1) shuttles between the nucleus and cytoplasm until G_2 , when it accumulates in the nucleus just prior to the onset of mitosis (Figure 14.7). According to one proposal, nuclear accumulation of cyclin B1 is facilitated by phosphorylation of one or more serine residues that reside in its nuclear export signal (NES, page 492). In this model, phosphorylation blocks subsequent export of the cyclin back to the cytoplasm. According to an alternate proposal, cyclin B1–Cdk1 stimulates its own translocation into the nucleus by phosphorylating and activating components of the nuclear import machinery. Regardless of the mechanism, if nuclear accumulation of the cyclin is blocked, cells fail to initiate cell division.

As noted above, the proteins and processes that control the cell cycle are remarkably conserved among eukaryotes. As in yeast, successive waves of synthesis and degradation of different cyclins play a key role in driving mammalian cells from one stage to the next. Unlike yeast cells, which have a single Cdk, mammalian cells produce several different versions of this protein kinase. Different cyclin–Cdk complexes target different groups of substrates at different points within the cell cycle. The pairing between individual cyclins and Cdks is specific, and only certain combinations are found (Figure 14.8a). In mammalian cells, for example, the activity of a cyclin E–Cdk2 complex drives the cell into S phase, whereas activity of a cyclin B1–Cdk1 complex (the mammalian MPF)

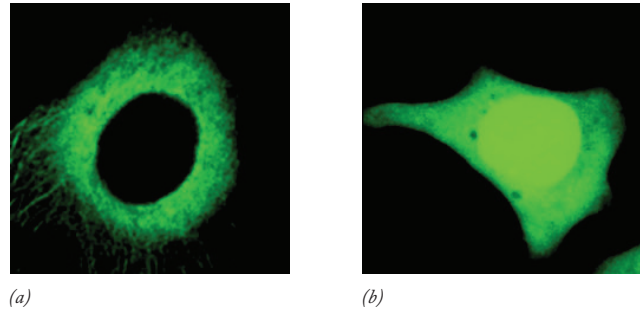


Figure 14.7 Experimental demonstration of subcellular localization during the cell cycle. Micrographs of a living HeLa cell that has been injected with cyclin B1 linked to the green fluorescent protein (page 273). The cell shown in *a* is in the G_2 phase of its cell cycle, and the fluorescently labeled cyclin B1 is localized almost entirely in the cytoplasm. The micrograph in *b* shows the cell in prophase of mitosis, and the labeled cyclin B1 is concentrated in the cell nucleus. The basis for this change in localization is discussed in the text. (FROM PAUL CLUTE AND JONATHAN PINES, NATURE CELL BIOL. 1:83, 1999. REPRINTED BY PERMISSION FROM MACMILLAN PUBLISHERS LIMITED.)

drives the cell into mitosis. Cdks do not always stimulate activities, but can also inhibit inappropriate events. For example, cyclin B1–Cdk1 activity during G_2 prevents a cell from rereplicating DNA that has already been replicated earlier in the cell cycle (page 560). This helps ensure that each region of the genome is replicated once and only once per cell cycle.

The roles of the various cyclin–Cdk complexes shown in Figure 14.8a have been determined by a wide range of biochemical studies carried out on mammalian cells for more than two decades. Over the past few years, the roles of these proteins have been reexamined in knockout mice, with some surprising results (Figure 14.8b). As expected, the phenotype of a particular knockout mouse depends on the gene that has been eliminated. Mice that are unable to synthesize Cdk1, cyclin B1, cyclins E1 and E2, or cyclin A2, die as early embryos, suggesting that the proteins encoded by these genes are essential for a normal cell cycle. In contrast, a mouse embryo that lacks the genes encoding *all* of the other cell cycle Cdks (namely, Cdks 2, 4, and 6) is capable of developing to a stage with fully formed organs, although the animal does not survive to birth (Figure 14.8b). Cells taken from such embryos are capable of proliferating in culture, though more slowly than normal cells. This finding indicates that, as in yeast, Cdk1 is the only Cdk *required* to drive a mammalian cell through all of the stages of the cell cycle. In other words, even though the other Cdks are normally expressed at specific times during the mammalian cell cycle, Cdk1 is able to “cover” for their absence, ensuring that all of the required substrates are phosphorylated at each stage of the cell cycle. This is a classical case of *redundancy*, in which a protein is able to carry out functions that it would not normally perform. Still, the absence of one of these “nonessential” cyclins or Cdks typically results in distinct cell cycle abnormalities, at least in certain types of cells. Mice lacking a gene for cyclin D1, for example, are smaller than control animals, which stems from a reduction in the level of cell division throughout the body. In addition, cyclin D1-deficient animals display a

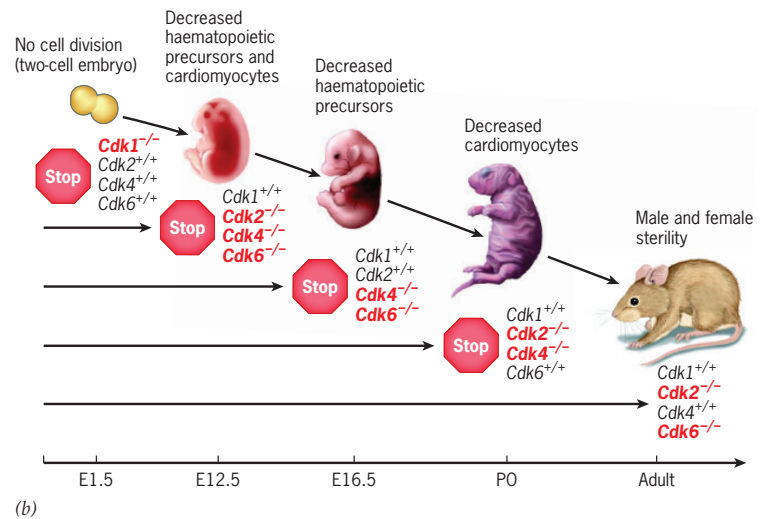
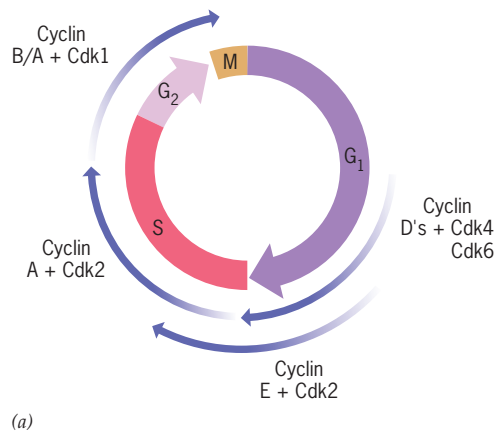


Figure 14.8 Cyclin-Cdks in the mammalian cell cycle.

(a) Combinations between various cyclins and cyclin-dependent kinases at different stages in the mammalian cell cycle. Cdk activity during early G_1 is very low, which promotes the formation of prereplication complexes at the origins of replication (see Figure 13.20). By mid- G_1 , Cdk activity is evident due to the association of Cdk4 and Cdk6 with the D-type cyclins (D1, D2, and D3). Among the substrates for these Cdks is an important regulatory protein called pRb (Section 16.3, Figure 16.12). The phosphorylation of pRb leads to the transcription of a number of genes, including those that code for cyclins E and A, Cdk1, and proteins involved in replication. The G_1 -S transition, which includes the initiation of replication, is driven by the activity of the cyclin E-Cdk2 and cyclin A-Cdk2 complexes. The transition from G_2 to M and passage through early M is driven by the sequential activity of cyclin A-Cdk1 and cyclin B1-Cdk1 complexes, which phosphorylate such diverse substrates as cytoskeletal proteins, histones, and proteins of the nuclear envelope. (The mammalian Cdk1 kinase is equivalent to the fission yeast *cdc2* kinase, and its inhibition and activation are similar to

that indicated in Figure 14.6.) (b) The effects on mouse development of the deletion of genes (shown in red) encoding various Cdks. Of the four primary mammalian Cdks, only Cdk1 is absolutely required for cell division. Embryos that express only Cdk1 die during the course of embryonic development. Mice expressing both Cdk1 and Cdk4 develop into adults that are sterile, owing to defects in the meiotic cell cycles. E, embryonic day number; P, postnatal day number. (A: C. G. SHERR, CELL 73:1060, 1993; CELL BY CELL PRESS. REPRODUCED WITH PERMISSION OF CELL PRESS IN THE FORMAT REUSE IN A BOOK/ TEXTBOOK VIA COPYRIGHT CLEARANCE CENTER. H. A. COLLIER, NATURE REV. MOL. CELL BIOL. 8:667, 2007. NATURE REVIEWS MOLECULAR CELL BIOLOGY BY NATURE PUBLISHING GROUP. REPRODUCED WITH PERMISSION OF NATURE PUBLISHING GROUP IN THE FORMAT REUSE IN A BOOK/ TEXTBOOK VIA COPYRIGHT CLEARANCE CENTER. B: MALUMBERS AND BARBACID, NAT REV. CANCER 9, 160, 2009 FIGURE 2. NATURE REVIEWS CANCER BY NATURE PUBLISHING GROUP. REPRODUCED WITH PERMISSION OF NATURE PUBLISHING GROUP IN THE FORMAT REUSE IN A BOOK/TEXTBOOK VIA COPYRIGHT CLEARANCE CENTER.)

particular lack of cell proliferation during development of the retina. Mice lacking Cdk4 develop without insulin-producing cells in their pancreas. Mice lacking Cdk2 appear to develop normally but exhibit specific defects during meiosis (Figure 14.8b), which reinforces the important differences in the regulation of mitotic and meiotic divisions.

Checkpoints, Cdk Inhibitors, and Cellular Responses

Ataxia-telangiectasia (AT) is an inherited recessive disorder characterized by a host of diverse symptoms, including a greatly increased risk for certain types of cancer.² During the late 1960s—following the deaths of several individuals undergoing radiation therapy—it was discovered that patients with AT are extremely sensitive to ionizing radiation (page 567). So too are cells from these patients, which lack a crucial protective response found in normal cells. When normal cells are subjected to treatments that damage DNA, such as ionizing radiation or DNA-altering drugs, their progress through the cell cycle stops while the damage is repaired. If, for example, a

normal cell is irradiated during the G_1 phase of the cell cycle, it delays progression into S phase. Similarly, cells irradiated in S phase delay further DNA synthesis, whereas cells irradiated in G_2 delay entry into mitosis.

Studies of this type carried out in yeast gave rise to a concept, formulated by Leland Hartwell and Ted Weinert in 1988, that cells possess **checkpoints** as part of their cell cycle. Checkpoints are surveillance mechanisms that halt the progress of the cell cycle if (1) any of the chromosomal DNA is damaged, or (2) certain critical processes, such as DNA replication during S phase or chromosome alignment during M phase, have not been properly completed. Checkpoints ensure that each of the various events that make up the cell cycle occurs accurately and in the proper order. Many of the proteins of the checkpoint machinery have no role in normal cell cycle events and are only called into action when an abnormality appears. In fact, the genes encoding several checkpoint proteins were first identified in mutant yeast cells that continued their progress through the cell cycle, despite suffering DNA damage or other abnormalities that caused serious defects.

Checkpoints are activated throughout the cell cycle by a system of sensors that recognize DNA damage or cellular abnormalities. If a sensor detects the presence of a defect, it triggers a response that temporarily arrests further cell cycle

²Other symptoms of the disease include unsteady posture (ataxia) resulting from degeneration of nerve cells in the cerebellum, permanently dilated blood vessels (telangiectasia) in the face and elsewhere, susceptibility to infection, and cells with an abnormally high number of chromosome aberrations. The basis for the first two symptoms has yet to be determined.

progress. The cell can then use the delay to repair the damage or correct the defect rather than continuing to the next stage. This is especially important because mammalian cells that undergo division with genetic damage run the risk of becoming transformed into a cancer cell. If the DNA is damaged beyond repair, the checkpoint mechanism can transmit a signal that leads either to (1) the death of the cell or (2) its conversion to a state of permanent cell cycle arrest (known as senescence).

We have seen in numerous places in this text where the study of a rare human disease has led to a discovery of basic importance in cell and molecular biology. The cell's DNA damage response provides another example of this path to discovery. The gene responsible for ataxia-telangiectasia (the *ATM* gene) encodes a protein kinase that is activated by certain DNA lesions, particularly double-stranded breaks (page 567). Remarkably, the presence of a single break in one of the cell's DNA molecules is sufficient to cause rapid, large-scale activation of ATM molecules, causing cell cycle arrest. A related protein kinase called ATR is also activated by DNA breaks as well as other types of lesions, including those resulting from incompletely replicated DNA or UV irradiation. Both ATM and ATR are part of multiprotein complexes capable of binding to chromatin that contains damaged DNA. Once bound, ATM and ATR can phosphorylate a remarkable variety of proteins that participate in cell cycle checkpoints and DNA repair.

How does a cell stop its progress from one stage of the cell cycle to the next? We will briefly examine two well-studied pathways available to mammalian cells to arrest their cell cycle in response to DNA damage.

1. If a cell preparing to enter mitosis is subjected to UV irradiation, ATR kinase is activated and the cell arrests in G₂. ATR kinase molecules are thought to be recruited to sites of protein-coated, single-stranded DNA (step 1, Figure 14.9), such as those present as UV-damaged DNA is repaired (Figure 13.25). ATR phosphorylates and activates a checkpoint kinase, called Chk1 (step 2), which in turn phosphorylates Cdc25 on a particular serine residue (step 3), making the Cdc25 molecule a target for a special adaptor protein that binds to Cdc25 in the cytoplasm (steps 4, 5). This interaction inhibits Cdc25's phosphatase activity and prevents it from being reimported into the nucleus. As discussed on page 577, Cdc25 normally plays a key role in the G₂/M transition by removing inhibitory phosphates from Cdk1. Thus, the absence of Cdc25 from the nucleus leaves the Cdk in an inactive state (step 6) and the cell arrested in G₂.
2. Damage to DNA also leads to the synthesis of proteins that directly inhibit the cyclin–Cdk complex that drives the cell cycle. For example, cells exposed to ionizing radiation in G₁ synthesize a protein called p21 (molecular mass of 21 kDa) that inhibits the kinase activity of the G₁ Cdk. This prevents the cells from phosphorylating key substrates and from entering S phase. ATM is involved in this checkpoint mechanism. In this particular DNA-damage response, the breaks in DNA that are caused by ionizing radiation serve as sites for the recruitment of a protein complex termed MRN (step a, Figure 14.9). MRN can be

considered as a sensor of DNA breaks. MRN recruits and activates ATM, which phosphorylates and activates another checkpoint kinase called Chk2 (step b). Chk2 in turn phosphorylates a transcription factor (p53) (step c),

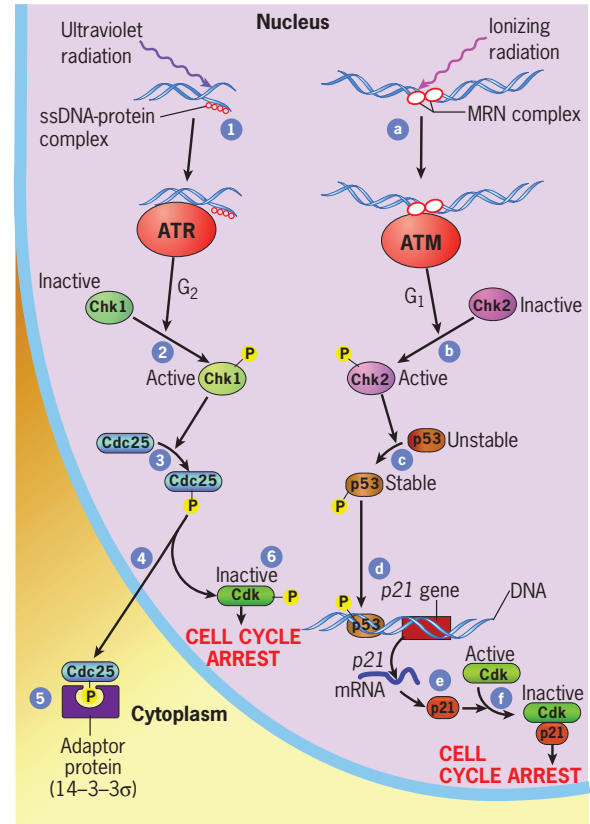


Figure 14.9 Models for the mechanism of action of two DNA-damage checkpoints. ATM and ATR are protein kinases that become activated following specific types of DNA damage. Each of these proteins acts through checkpoint signaling pathways that lead to cell cycle arrest. ATM becomes activated in response to double-strand breaks, which are detected by the MRN protein complex (step a). ATR, on the other hand, becomes activated by protein-coated ssDNA (step 1) that forms when replication forks become stalled or the DNA is being repaired after various types of damage. In the G₂ pathway shown here, ATR phosphorylates and activates the checkpoint kinase Chk1 (step 2), which phosphorylates and inactivates the phosphatase Cdc25 (step 3), which normally shuttles between the nucleus and cytoplasm (step 4). Once phosphorylated, Cdc25 is bound by an adaptor protein in the cytoplasm (step 5) and cannot be reimported into the nucleus, which leaves the Cdk in its inactivated, phosphorylated state (step 6). In the G₁ pathway shown here, ATM phosphorylates and activates the checkpoint kinase Chk2 (step b), which phosphorylates p53 (step c). p53 is normally very short-lived, but phosphorylation by Chk2 stabilizes the protein, enhancing its ability to activate *p21* transcription (step d). Once transcribed and translated (step e), p21 directly inhibits the Cdk (step f). Many other proteins, including histone-modifying enzymes, chromatin remodeling complexes, and histone variants are involved in mediating the response to DNA damage but are not discussed (see *Curr. Opin. Cell Biol.* 21:245, 2009; *Nature Revs. Mol. Cell Biol.* 10:243, 2009; *Nature Cell Biol.* 13:1161, 2011; and *Genes Develop.* 25:409, 2011).



Figure 14.10 p27: a Cdk inhibitor that arrests cell cycle progression. (a) Three-dimensional structure of a complex between p27 and cyclin A–Cdk2. Interaction with p27 alters the conformation of the Cdk catalytic subunit, inhibiting its protein kinase activity. (b) A pair of littermates at 12 weeks of age. In addition to possessing different genes for coat color, the mouse with dark fur has been genetically engineered to lack both copies of the *p27* gene (denoted as *p27*^{-/-}), which accounts for its larger size. (c) Comparison of the thymus glands

from a normal (left) and a *p27*^{-/-} mouse (right). The gland from the *p27* knockout mouse is much larger owing to an increased number of cells. (A: FROM ALICIA A. RUSSO ET AL., NATURE 382:327, 1996, FIG. 2A. COURTESY OF NIKOLA PAVLETICH, HOWARD HUGHES MEDICAL INSTITUTE; REPRINTED BY PERMISSION OF MACMILLAN PUBLISHERS LIMITED; B,C: FROM KEIKO NAKAYAMA ET AL., COURTESY OF KEI-ICHI NAKAYAMA, CELL 85:710, 711, 1996; WITH PERMISSION FROM ELSEVIER.)

which leads to the transcription and translation of the *p21* gene (steps d and e) and subsequent inhibition of Cdk (step f). Approximately 50 percent of all human tumors show evidence of mutations in the gene that encodes p53, which reflects its importance in the control of cell growth. The role of p53 is discussed at length in Chapter 16.

p21 is only one of at least seven known Cdk inhibitors. The interaction between a related Cdk inhibitor (p27) and one of the cyclin–Cdk complexes is shown in Figure 14.10a. In this structural model, the p27 molecule drapes itself across both subunits of the cyclin A–Cdk2 complex, changing the conformation of the catalytic subunit and inhibiting its kinase activity. In many cells, p27 must be phosphorylated and then degraded before progression into S phase can occur.

Cdk inhibitors, such as p21 and p27, are also active in cell differentiation. Just before cells begin to differentiate—whether into muscle cells, liver cells, blood cells, or some other type—they typically withdraw from the cell cycle and stop dividing. Cdk inhibitors are thought to either allow or directly induce cell cycle withdrawal. Just as the functions of specific Cdks and cyclins have been studied in knockout mice, so too have their inhibitors. Knockout mice that lack the *p27* gene show a distinctive phenotype: they are larger than normal (Figure 14.10b), and certain organs, such as the thymus gland and spleen, contain a significantly greater number of cells than those of a normal animal (Figure 14.10c). In normal mice, the cells of these particular organs synthesize relatively high levels of p27, and it is presumed that the absence of this protein in the *p27*-deficient animals allows the cells to divide several more times before they differentiate.

REVIEW

1. What is the cell cycle? What are the stages of the cell cycle? How does the cell cycle vary among different types of cells?

2. Describe how [³H]thymidine and autoradiography can be used to determine the length of the various periods of the cell cycle.
3. What is the effect of fusing a G₁-phase cell with one in M; of fusing a G₂- or S-phase cell with one in M?
4. How does the activity of MPF vary throughout the cell cycle? How is this correlated with the concentration of cyclins? How does the cyclin concentration affect MPF activity?
5. What are the respective roles of CAK, Wee1, and Cdc25 in controlling Cdk activity in fission yeast cells? What is the effect of mutations in the *wee1* or *cdc25* genes in these cells?
6. What is meant by a cell cycle checkpoint? What is its importance? How does a cell stop its progress at one of these checkpoints?

14.2 | M Phase: Mitosis and Cytokinesis



Whereas our understanding of cell cycle regulation rests largely on genetic studies in yeast, our knowledge of M phase is based on more than a century of microscopic and biochemical research on animals and plants. The name “mitosis” comes from the Greek word *mitos*, meaning “thread.” The name was coined in 1882 by the German biologist Walther Flemming to describe the thread-like chromosomes that mysteriously appeared in animal cells just before they divided in two. The beauty and precision of cell division is best appreciated by watching a time-lapse video of the process (e.g., www.bio.unc.edu/faculty/salmon/lab/mitosis/mitosismovies.html) rather than reading about it in a textbook.

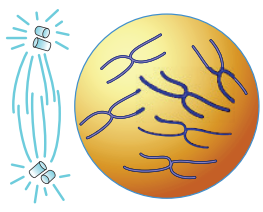

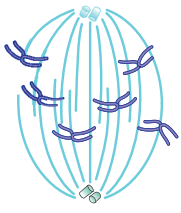
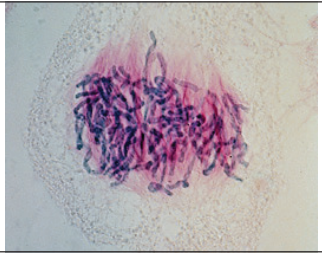
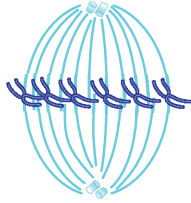

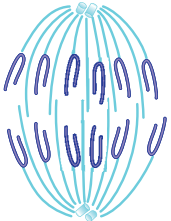
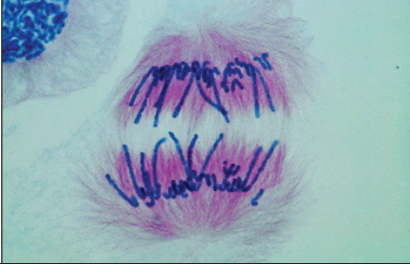
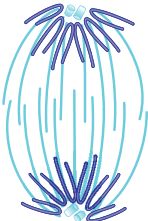
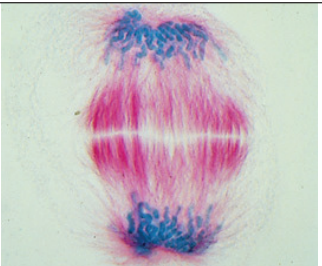
Prophase		
<ol style="list-style-type: none"> 1. Chromosomal material condenses to form compact mitotic chromosomes. Chromosomes are seen to be composed of two chromatids attached together at the centromere. 2. Cytoskeleton is disassembled, and mitotic spindle is assembled. 3. Golgi complex and ER fragment. Nuclear envelope disperses. 		
Prometaphase		
<ol style="list-style-type: none"> 1. Chromosomal microtubules attach to kinetochores of chromosomes. 2. Chromosomes are moved to spindle equator. 		
Metaphase		
<ol style="list-style-type: none"> 1. Chromosomes are aligned along metaphase plate, attached by chromosomal microtubules to both poles. 		
Anaphase		
<ol style="list-style-type: none"> 1. Centromeres split, and chromatids separate. 2. Chromosomes move to opposite spindle poles. 3. Spindle poles move farther apart. 		
Telophase		
<ol style="list-style-type: none"> 1. Chromosomes cluster at opposite spindle poles. 2. Chromosomes become dispersed. 3. Nuclear envelope assembles around chromosome clusters. 4. Golgi complex and ER reforms. 5. Daughter cells formed by cytokinesis. 		



Figure 14.11 The stages of mitosis in an animal cell (left drawings) and a plant cell (right photos).
(MICROGRAPHS COURTESY OF ANDREW BAJER.)

Mitosis is a process of nuclear division in which the replicated DNA molecules of each chromosome are faithfully segregated into two nuclei. Mitosis is usually accompanied by **cytokinesis**, a process by which a dividing cell splits in two, partitioning the cytoplasm into two cellular packages. The two daughter cells resulting from mitosis and cytokinesis possess a genetic content identical to each other and to the mother cell from which they arose. Mitosis, therefore, maintains the chromosome number and generates new cells for the growth and maintenance of an organism. Mitosis can take place in either haploid or diploid cells. Haploid mitotic cells are found in fungi, plant gametophytes, and a few animals (including male bees known as drones). Mitosis is a stage of the cell cycle when the cell devotes virtually all of its energy to a single activity—chromosome segregation. As a result, most metabolic activities of the cell, including transcription and translation, are curtailed during mitosis, and the cell becomes relatively unresponsive to external stimuli.

We have seen in previous chapters how much can be learned about the factors responsible for a particular process by studying that process outside of a living cell (page 276). Our understanding of the biochemistry of mitosis has been greatly aided by the use of extracts prepared from frog eggs. These extracts contain stockpiles of all the materials (histones, tubulin, etc.) necessary to support mitosis. When chromatin or whole nuclei are added to the egg extract, the chromatin is compacted into mitotic chromosomes, which are segregated by a mitotic spindle that assembles spontaneously within the cell-free mixture. In many experiments, the role of a particular protein in mitosis can be studied by removing that protein from the egg extract by addition of an antibody (immunodepletion) and determining whether the process can continue in the absence of that substance (see Figure 14.21 for an example).

Mitosis is generally divided into five stages (Figure 14.11), *prophase*, *prometaphase*, *metaphase*, *anaphase*, and *telophase*, each characterized by a particular series of events. Keep in mind that each of these stages represents a segment of a continuous process; the division of mitosis into arbitrary phases is done only for the sake of discussion and experimentation.

Prophase

During the first stage of mitosis, that of **prophase**, the duplicated chromosomes are prepared for segregation and the mitotic machinery is assembled.

Formation of the Mitotic Chromosome The nucleus of an interphase cell contains tremendous lengths of chromatin fibers. The extended state of interphase chromatin is ideally suited for the processes of transcription and replication but not for segregation into two daughter cells. Before segregating its chromosomes, a cell converts them into much shorter, thicker structures by a remarkable process of **chromosome compaction** (or *chromosome condensation*), which occurs during early prophase (Figures 14.11 and 14.12).

As described on page 496, the chromatin of an interphase cell is organized into fibers approximately 30 nm in diameter.

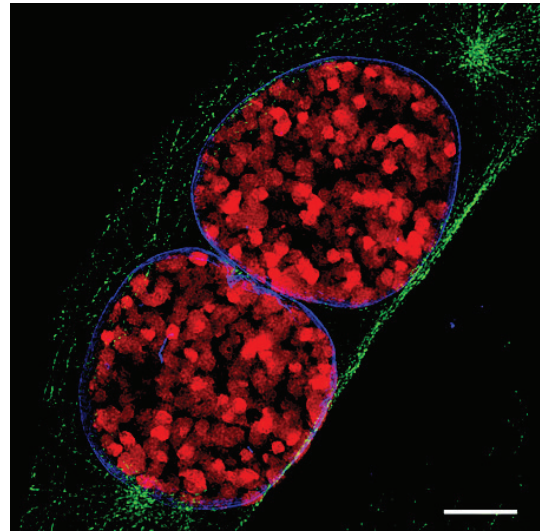


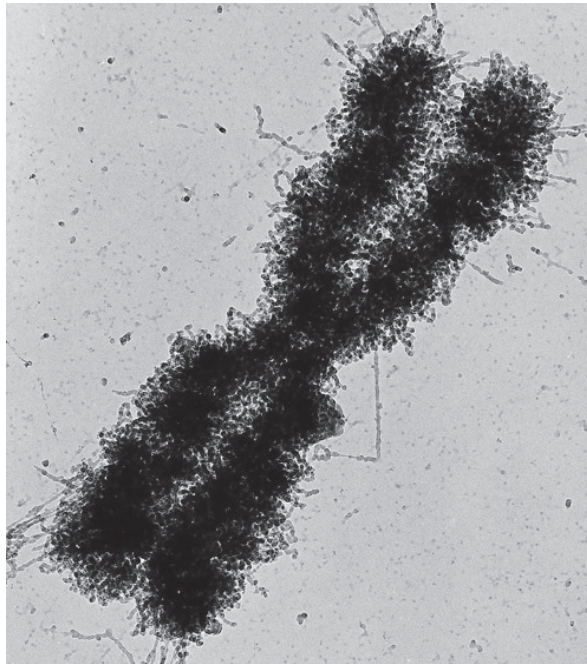
Figure 14.12 Prophase nuclear morphology. Light-optical section through two mouse cell nuclei in prophase, recorded with super-resolution 3D-structured illumination microscopy (3D-SIM). Condensed chromosomes are shown in red, the nuclear envelope in blue and microtubules, in green. Scale bar is 5 μm . (COURTESY OF LOTHAR SCHERMELLEH.)

Although there is debate on this issue, mitotic chromosomes are thought to be composed of similar types of fibers as seen by electron microscopic examination of whole chromosomes isolated from mitotic cells (Figure 14.13a). According to this viewpoint, chromosome compaction does not alter the nature of the chromatin fiber, but rather the way that the chromatin fiber is packaged. Treatment of mitotic chromosomes with solutions that solubilize the histones and the majority of the nonhistone proteins reveals a structural framework or scaffold that retains the basic shape of the intact chromosome (Figure 14.13b). Loops of DNA are attached at their base to the nonhistone proteins that make up this chromosome scaffold (shown at higher magnification in Figure 12.15).

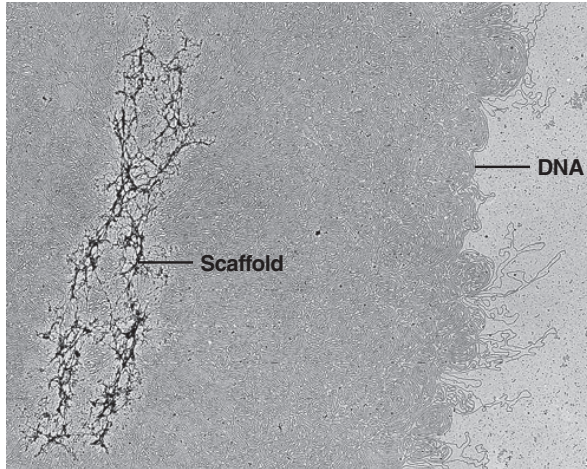
Research on chromosome compaction has focused on an abundant multiprotein complex called *condensin*. The proteins of condensin were discovered by incubating nuclei in frog egg extracts and identifying those proteins that associated with the chromosomes as they underwent compaction. Removal of condensin from the extracts prevented normal chromosome compaction. How is condensin involved in such dramatic changes in chromatin architecture? There is very little data available from *in vivo* studies to answer this question, but there is considerable speculation.

Supercoiled DNA occupies a much smaller volume than relaxed DNA (see Figure 10.12), and studies suggest that DNA supercoiling plays a key role in compacting a chromatin fiber into the tiny volume occupied by a mitotic chromosome. In the presence of a topoisomerase and ATP, condensin is able to bind to DNA *in vitro* and curl the DNA into positively supercoiled loops. This finding fits nicely with the observation that chromosome compaction at prophase requires topoisomerase.

merase II, which along with condensin is present as part of the mitotic chromosome scaffold (Figure 14.13*b*). A speculative model for condensin action is shown in Figure 14.14. Con-



(a)



(b)

Figure 14.13 The mitotic chromosome. (a) Electron micrograph of a whole-mount preparation of a human mitotic chromosome. The structure is seen to be composed of a knobby fiber 30 nm in diameter, which is similar to that found in interphase chromosomes. (b) Appearance of a mitotic chromosome after the histones and most of the non-histone proteins have been removed. The residual proteins form a scaffold from which loops of DNA are seen to emerge (the DNA loops are shown more clearly in Figure 12.15). (A: COURTESY OF GUNTHER F. BAHR, ARMED FORCES INSTITUTE OF PATHOLOGY, WASHINGTON, D.C.; B: FROM JAMES R. PAULSON AND ULRICH K. LAEMMLI, CELL 12:820, 1977, WITH PERMISSION FROM ELSEVIER.)

densin is activated at the onset of mitosis by phosphorylation of several of its subunits by the cyclin-Cdk responsible for driving cells from G_2 into mitosis. Thus condensin is one of the targets through which Cdks are able to trigger cell cycle activities. The subunit structure of a V-shaped condensin molecule is shown in the right inset of Figure 14.14.

As the result of compaction, the chromosomes of a mitotic cell appear as distinct, rod-like structures. Close examination of mitotic chromosomes reveals each of them to be composed of two mirror-image, “sister” **chromatids** (Figure 14.13*a*). Sister chromatids are a result of replication in the previous interphase.

Prior to replication, the DNA of each interphase chromosome becomes associated at sites along its length with a multiprotein complex called **cohesin** (Figure 14.14). Following replication, cohesin holds the two sister chromatids together continuously through G_2 and into mitosis when they are ulti-

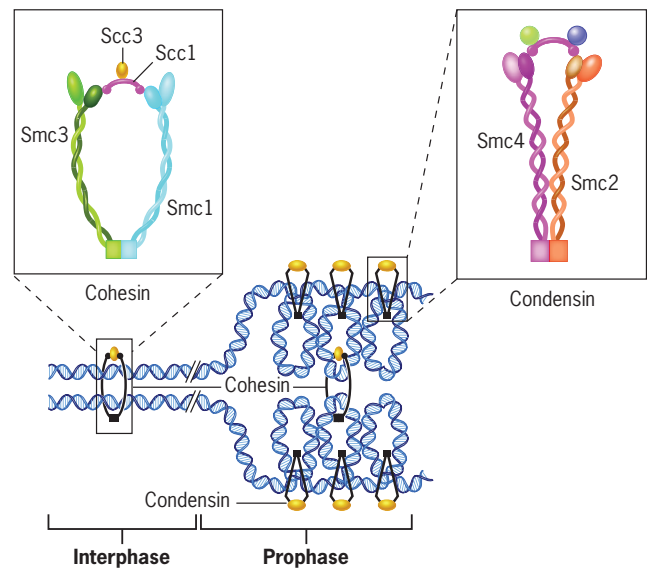
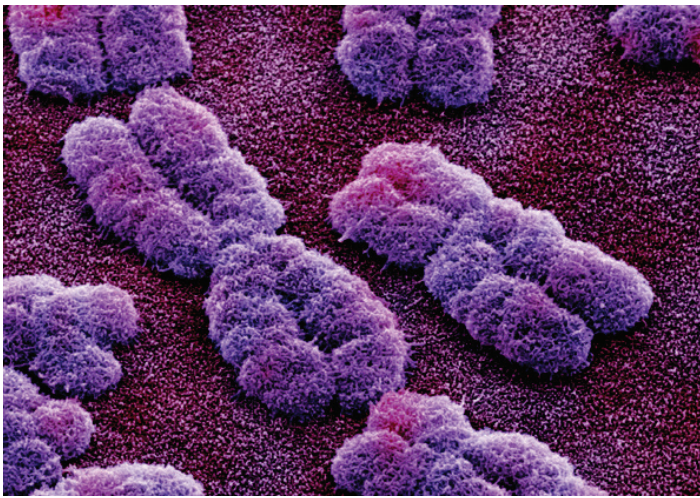
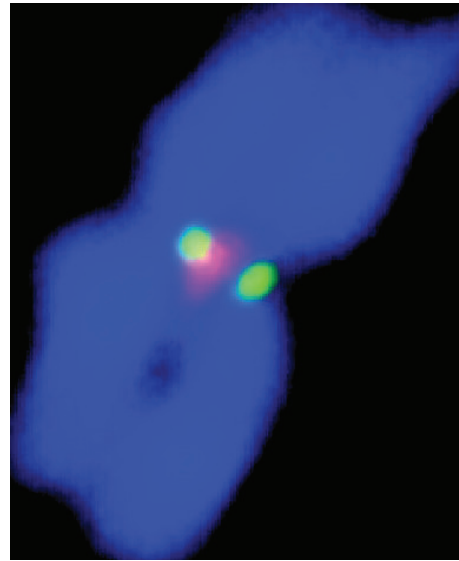


Figure 14.14 Model for the roles of condensin and cohesin in the formation of mitotic chromosomes. Just after replication, the DNA helices of a pair of sister chromatids would be held in association by cohesin molecules that encircled the sister DNA helices, as shown at the left of the drawing. As the cell entered mitosis, the compaction process would begin, aided by condensin molecules, as shown in the right part of the drawing. In this model, condensin brings about chromosome compaction by forming a ring around supercoiled loops of DNA within chromatin. Cohesin molecules would continue to hold the DNA of sister chromatids together. It is proposed (but not shown in this drawing), that cooperative interactions between condensin molecules would then organize the supercoiled loops into larger coils, which are then folded into a mitotic chromosome fiber. The top left and right insets show the subunit structure of an individual cohesin and condensin complex, respectively. Both complexes are built around a pair of SMC subunits. Each of the SMC polypeptides folds back on itself to form a highly elongated antiparallel, coiled coil with an ATP-binding globular domain where the N- and C-termini come together. Cohesin and condensin also have two or three non-SMC subunits that complete the ring-like structure of these proteins.



(a)



(b)

Figure 14.15 Each mitotic chromosome is comprised of a pair of sister chromatids connected to one another by the protein complex **cohesin**. (a) Colorized scanning electron micrograph of several metaphase chromosomes showing the paired identical chromatids associated loosely along their length and joined tightly at the centromere. The chromatids are not split apart from one another until anaphase. (b) Fluorescence micrograph of a metaphase chromosome in a cultured

human cell. The DNA is stained blue, the kinetochores are green, and cohesin is red. At this stage of mitosis, cohesin has been lost from the arms of the sister chromatids but remains concentrated at the centromeres where the two sisters are tightly joined. (A: ANDREW SYRED/PHOTO RESEARCHERS, INC.; B: BY S. HAUF AND JAN-MICHAEL PETERS, NATURE CELL BIOL. 3:E17, 2001 FIG. 1C. REPRINTED BY PERMISSION FROM MACMILLAN PUBLISHERS LIMITED.)

mately separated. As indicated in the insets of Figure 14.14, condensin and cohesin have a similar structural organization. A number of experiments support the hypothesis that the cohesin ring encircles two sister DNA molecules as shown in both the left and right portions of Figure 14.14.

In vertebrates, cohesin is released from the chromosomes in two distinct stages. Most of the cohesin dissociates from the arms of the chromosomes as they become compacted during prophase. Dissociation is induced by phosphorylation of cohesin subunits by two important mitotic enzymes called Polo-like kinase and Aurora B kinase. In the wake of this event, the chromatids of each mitotic chromosome are held relatively loosely along their extended arms, but much more tightly at their centromeres (Figure 14.13a and Figure 14.15). Cohesin remains at the centromeres because of the presence there of a phosphatase that removes any phosphate groups added to the protein by the kinases. Release of cohesin from the centromeres is normally delayed until anaphase as described on page 592. If the phosphatase is experimentally inactivated, sister chromatids separate from one another prematurely prior to anaphase.

Centromeres and Kinetochores The most notable landmark on a mitotic chromosome is an indentation or *primary constriction*, which marks the position of the centromere (Figure 14.13a). The centromere is the residence of highly repeated DNA sequences (see Figure 10.19) that serve as the binding sites for specific proteins. Examination of sections through a

mitotic chromosome reveals the presence of a proteinaceous, button-like structure, called the **kinetochore**, at the outer surface of the centromere of each chromatid (Figure 14.16a,b). Most of the proteins that make up the kinetochore assemble at the centromere at early prophase. Kinetochore proteins are thought to be recruited to the centromere because of the presence there of the novel nucleosomes containing the histone variant CENP-A (page 509). As will be apparent shortly, the kinetochore functions as (1) the site of attachment of the chromosome to the dynamic microtubules of the mitotic spindle (as in Figure 14.30), (2) the residence of several motor proteins involved in chromosome motility (Figure 14.16c), and (3) a key component in the signaling pathway of an important mitotic checkpoint (see Figure 14.31).

A question of great interest to scientists studying kinetochores is how these structures are able to maintain their attachment to microtubules that are continually growing and shrinking at their plus end. To maintain this type of “floating grip,” the coupler would have to move with the end of the microtubule as subunits were added or removed. Figure 14.16c depicts two types of proteins that have been implicated as possible linkers of a kinetochore to dynamic microtubules, namely, motor proteins and a rod-shaped protein complex called Ndc80. Ndc80 is an essential kinetochore component that forms fibrils that appear to reach out and bind the surface of the adjacent microtubule. Cells lacking any of the four proteins that make up the Ndc80 complex exhibit severe spindle attachment defects.

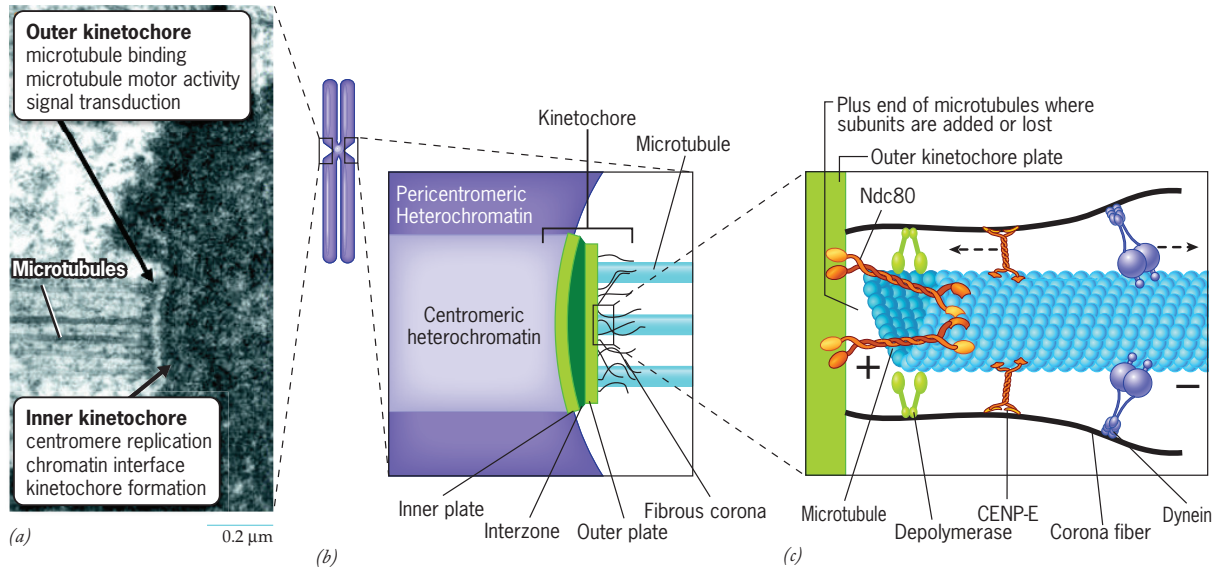


Figure 14.16 The kinetochore. (a) Electron micrograph of a section through one of the kinetochores of a mammalian metaphase chromosome, showing its three-layered (trilaminar) structure. Microtubules of the mitotic spindle can be seen to terminate at the kinetochore. (b) Schematic representation of the kinetochore, which contains an electron-dense inner and outer plate separated by a lightly staining interzone. Proposed functions of the inner and outer plates are indicated in part *a*. The inner plate contains a variety of proteins attached to the centromeric heterochromatin of the chromosome. Associated with the outer plate is the fibrous corona, which binds motor proteins involved in chromosome movement. (c) A schematic model showing a proposed disposition of several of the proteins found at the outer surface of the kinetochore. Among the motor proteins associated with the kinetochore, cytoplasmic dynein moves

toward the minus end of a microtubule, whereas CENP-E moves toward the plus end. These motors may also play a role in tethering the microtubule to the kinetochore. The protein labeled “depolymerase” is a member of the kinesin superfamily that functions in depolymerization of microtubules rather than motility. In this drawing, the depolymerases are in an inactive state (the microtubule is not depolymerizing). Ndc80 is a protein complex consisting of four different protein subunits that form a 57 nm-long, rod-shaped molecule extending outward from the body of the kinetochore. Globular domains at either end of the complex mediate attachment to the microtubule and kinetochore. These Ndc80 fibrils have been implicated as couplers of the kinetochore to the plus end of a dynamic microtubule. (A: FROM DON W. CLEVELAND, UCSD, ET AL., CELL 112:408, 2003 FIG. 1C, WITH PERMISSION FROM ELSEVIER. IMAGE COURTESY OF KEVIN SULLIVAN.)

Formation of the Mitotic Spindle We discussed in Chapter 9 how microtubule assembly in animal cells is initiated by a special microtubule-organizing structure, the **centrosome** (page 339). As a cell progresses past G_2 and into mitosis, the microtubules of the cytoskeleton undergo sweeping disassembly in preparation for their reassembly as components of a complex, micro-sized machine called the **mitotic spindle**. The rapid disassembly of the interphase cytoskeleton is thought to be accomplished by the inactivation of proteins that stabilize microtubules (e.g., microtubule-associated proteins, or MAPs) and the activation of proteins that destabilize these polymers.

To understand the formation of the mitotic spindle, we need to first examine the *centrosome cycle* as it progresses in concert with the cell cycle (Figure 14.17*a*). When an animal cell exits mitosis, the cytoplasm contains a single centrosome containing two centrioles situated at right angles to one another. Even before cytokinesis has been completed, the two centrioles of each daughter cell lose their close association to one another (they are said to “disengage”). This event is triggered by the enzyme separase, which becomes activated late in mitosis (page 592) and cleaves a proteinaceous link holding the centrioles together. Later, as DNA replication begins in the nucleus at the onset of S phase, each centriole of the centrosome initiates its “replication” in the cytoplasm. The process begins with the

appearance of a small *procentriole* next to each preexisting (maternal) centriole and oriented at right angles to it (Figure 14.17*b*). Subsequent microtubule elongation converts each procentriole into a full-length daughter centriole. At the beginning of mitosis, the centrosome splits into two adjacent centrosomes, each containing a pair of mother–daughter centrioles. The initiation of centrosome duplication at the G_1 –S transition is normally triggered by phosphorylation of a centrosomal protein by Cdk2, the same agent responsible for the onset of DNA replication (Figure 14.8). Centrosome duplication is a tightly controlled process so that each mother centriole produces only one daughter centriole during each cell cycle. The formation of additional centrioles can lead to abnormal cell division and may contribute to the development of cancer (Figure 14.17*c*).

The first stage in the formation of the mitotic spindle in a typical animal cell is the appearance of microtubules in a “sunburst” arrangement, or **aster** (Figure 14.18), around each centrosome during early prophase. As discussed in Chapter 9, microtubules grow by addition of subunits to their plus ends, while their minus ends remain associated with the pericentriolar material (PCM) of the centrosome (page 339). Phosphorylation of proteins of the PCM by Polo-like kinase is thought to play a key role in stimulating nucleation of spindle microtubules during prophase. The process of aster formation is

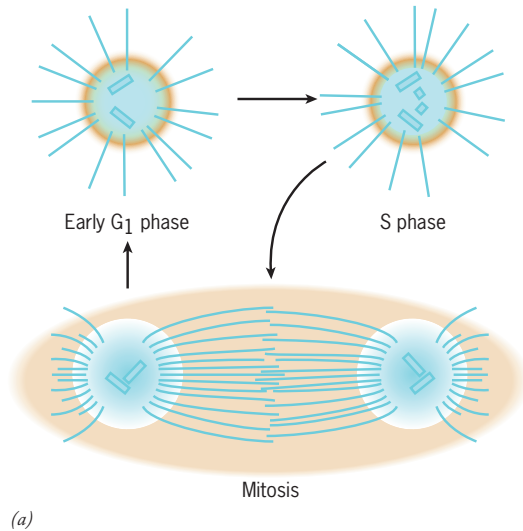


Figure 14.17 The centrosome cycle of an animal cell. (a) During G_1 , the centrosome contains a single pair of centrioles that are no longer as tightly associated as they were during mitosis. During S phase, daughter procentrioles form adjacent to maternal centrioles so that two pairs of centrioles become visible within the centrosome (see b). The daughter procentrioles continue to elongate during G_2 phase, and at the beginning of mitosis, the centrosome splits, with each pair of centrioles becoming part of its own centrosome. As they separate, the centrosomes organize the microtubule fibers that make up the mitotic spindle. (b) The centrosome of this cell is seen to contain two mother centrioles, each with an associated daughter procentriole (arrows). (c) This mouse mammary cancer cell contains more than the normal complement of two centrosomes (red) and has assembled a multipolar spindle apparatus (green). Additional centrosomes lead to chromosome missegregation and abnormal numbers of chromosomes (blue), which are characteristic of malignant cells. (A: AFTER D. R. KELLOGG ET AL., REPRODUCED WITH PERMISSION FROM THE ANNUAL REVIEW OF BIOCHEMISTRY, VOL. 63; COPYRIGHT 1994, ANNUAL REVIEW OF BIOCHEMISTRY BY ANNUAL REVIEWS. REPRODUCED WITH PERMISSION OF ANNUAL REVIEWS IN THE FORMAT REPUBLISH IN A BOOK VIA COPYRIGHT; B: FROM JEROME B. RATTNER AND STEPHANIE G. PHILLIPS, J. CELL BIOL. 57:363, 1973, FIG. 4. REPRODUCED WITH PERMISSION OF THE ROCKEFELLER UNIVERSITY PRESS; C: COURTESY OF THEA GOEPFERT AND W. R. BRINKLEY, BAYLOR COLLEGE OF MEDICINE, HOUSTON, TX.)

followed by separation of the centrosomes from one another and their subsequent movement around the nucleus toward opposite ends of the cell. Centrosome separation is driven by motor proteins associated with the adjacent microtubules. As the centrosomes separate, the microtubules stretching between them increase in number and elongate (Figure 14.18). Eventually, the two centrosomes reach points opposite one another, thus establishing the two poles of a *bipolar* mitotic spindle (as in Figure 14.17a). Following mitosis, one centrosome will be distributed to each daughter cell.

A number of different types of animal cells (including those of the early mouse embryo) lack centrosomes, as do the cells of higher plants, yet all of these cells construct a bipolar mitotic spindle and undergo a relatively typical mitosis. Functional mitotic spindles can even form in mutant *Drosophila* cells that lack centrosomes or in mammalian cells in which the centrosome has been experimentally removed. In all of these cases,

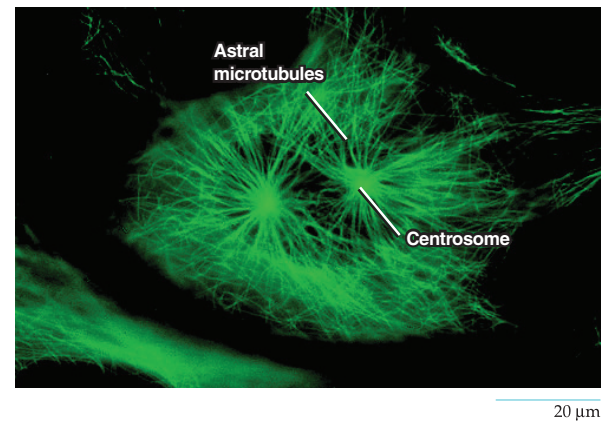
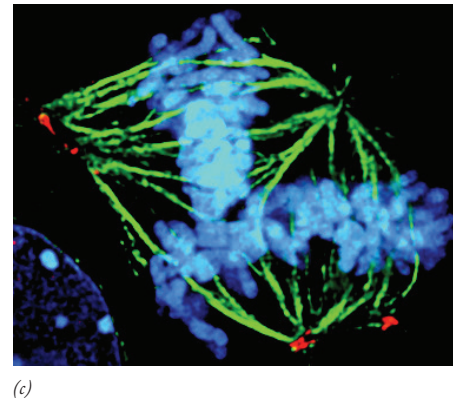
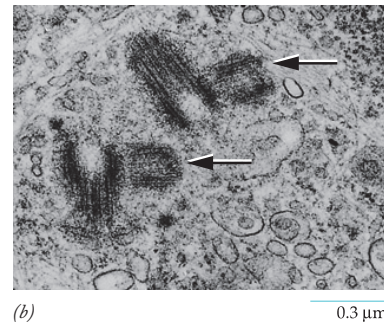


Figure 14.18 Formation of the mitotic spindle. During prophase, as the chromosomes are beginning to condense, the centrosomes move apart from one another as they organize the bundles of microtubules that form the mitotic spindle. This micrograph shows a cultured newt lung cell in early prophase that has been stained with fluorescent antibodies against tubulin, which reveals the distribution of the cell's microtubules (green). The microtubules of the developing mitotic spindle are seen to emanate as asters from two sites within the cell. These sites correspond to the locations of the two centrosomes that are moving toward opposite poles at this stage of prophase. The centrosomes are situated above the cell nucleus, which appears as an unstained dark region. (FROM JENNIFER WATERS, RICHARD W. COLE, AND CONLY L. RIEDER, J. CELL BIOL. 122:364, 1993, FIG. 2. REPRODUCED WITH PERMISSION OF THE ROCKEFELLER UNIVERSITY PRESS. COURTESY OF CONLY L. RIEDER.)

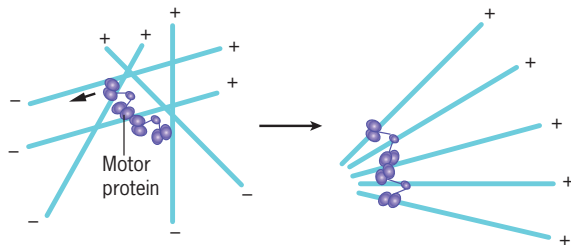


Figure 14.19 Formation of a spindle pole in the absence of centrosomes. In this model, each motor protein has multiple heads, which are bound to different microtubules. The movement of these motor proteins causes the minus ends of the microtubules to converge to form a distinct spindle pole. This type of mechanism is thought to facilitate the formation of spindle poles in the absence of centrosomes but may also play a role when centrosomes are present. (FROM A. A. HYMAN AND E. KARSENTI, CELL 84:406, 1996; BY PERMISSION OF CELL PRESS. CELL BY CELL PRESS. REPRODUCED WITH PERMISSION OF CELL PRESS IN THE FORMAT REUSE IN A BOOK/TEXTBOOK VIA COPYRIGHT CLEARANCE CENTER.)

the microtubules of the mitotic spindle are nucleated near the chromosomes rather than at the poles where centrosomes would normally reside. Then, once they have polymerized, the minus ends of the microtubules are brought together (i.e., focused) at each spindle pole through the activity of motor proteins (Figure 14.19). The chapter-opening photograph on page 572 shows a bipolar spindle that has formed in a frog egg extract through the activity of microtubule motors. These types of experiments suggested that cells possess two fundamentally different mechanisms—one centrosome-dependent and the other centrosome-independent—to achieve the same end result. Recent studies have indicated that both pathways to spindle formation operate simultaneously in the same cell and that even cells with functional centrosomes nucleate a significant fraction of their spindle microtubules at the chromosomes.

The Dissolution of the Nuclear Envelope and Partitioning of Cytoplasmic Organelles In most eukaryotic cells, the mitotic spindle is assembled in the cytoplasm and the chromosomes are compacted in the nucleoplasm. Interaction between the spindle and chromosomes is made possible by the breakdown of the nuclear envelope at the end of prophase. The three major components of the nuclear envelope—the nuclear pore complexes, nuclear lamina, and nuclear membranes—are disassembled in separate processes. All of these processes are thought to be initiated by phosphorylation of key substrates by mitotic kinases, particularly cyclin B–Cdk1. The nuclear pore complexes are disassembled as the interactions between nucleoporin subcomplexes are disrupted and the subcomplexes dissociate into the surrounding medium. The nuclear lamina is disassembled by depolymerization of the lamin filaments. The integrity of the nuclear membranes is first disrupted mechanically as holes are torn into the nuclear envelope by cytoplasmic dynein molecules associated with the outer nuclear membrane. The subsequent fate of the membranous portion of the nuclear envelope has been the subject of controversy. According to the classical view, the nuclear membranes are fragmented into a population of small vesicles that disperse throughout the mitotic cell. Alternatively, the membranes of the nuclear envelope may be absorbed into the membranes of the ER.

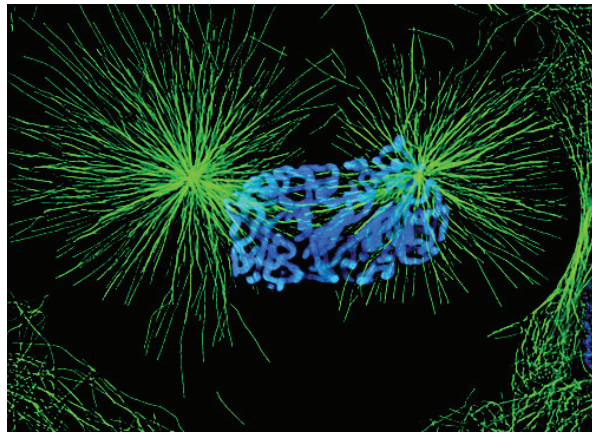
Some of the membranous organelles of the cytoplasm remain relatively intact through mitosis; these include mitochondria, lysosomes, and peroxisomes, as well as the chloroplasts of a plant cell. Considerable debate has been generated in recent years over the mechanism by which the Golgi complex and endoplasmic reticulum are partitioned during mitosis. According to one view, the contents of the Golgi complex become incorporated into the ER during prophase, and the Golgi complex ceases to exist briefly as a distinct organelle. According to an alternate view, the Golgi membranes become fragmented to form a distinct population of small vesicles that are partitioned between daughter cells. A third view based primarily on studies in algae and protists has the entire Golgi complex splitting in two, with each daughter cell receiving half of the original structure. Ultimately, we may learn that different types of cells or organisms utilize different mechanisms of Golgi inheritance. Our ideas about the fate of the ER have also changed. Recent studies on living, cultured mammalian cells suggest that the ER network remains relatively intact during mitosis. This view challenges earlier studies performed largely on eggs and embryos that suggested the ER undergoes extensive fragmentation during prophase.

Prometaphase

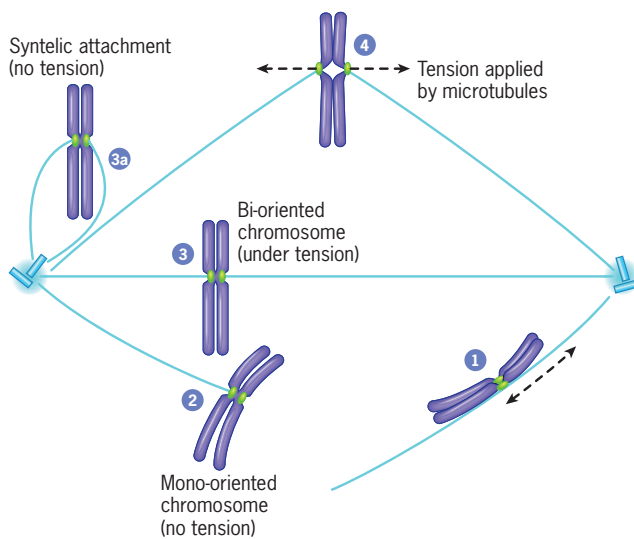
The dissolution of the nuclear envelope marks the start of the second phase of mitosis, **prometaphase**, during which mitotic spindle assembly is completed and the chromosomes are moved into position at the center of the cell. The following discussion provides a generalized picture of the steps of prometaphase; many variations on these events have been reported.

At the beginning of prometaphase, compacted chromosomes are scattered throughout the space that was the nuclear region (Figure 14.20a). As the microtubules of the spindle penetrate into the central region of the cell, the free (plus) ends of the microtubules are seen to grow and shrink in a dynamic fashion, as if they were “searching” for a chromosome. It is not certain whether searching is entirely random, as evidence suggests that microtubules may grow preferentially toward a site containing chromatin. Those microtubules that contact a kinetochore are “captured” and stabilized.

A kinetochore typically makes initial contact with the sidewall of a microtubule rather than its end (step 1, Figure 14.20b). Once initial contact is made, some chromosomes move actively along the wall of the microtubule, powered by motor proteins located in the kinetochore. Soon, however, the kinetochore tends to become stably associated with the plus end of one or more spindle microtubules from one of the spindle poles (step 2). A chromosome that is attached to microtubules from only one spindle pole represents an unstable intermediate stage in the course of prometaphase. Eventually, the unattached kinetochore on the sister chromatid captures its own microtubules from the opposite spindle pole (step 3). It has also been reported that unattached kinetochores serve as nucleating sites for the assembly of microtubules. These microtubules grow out from the chromosome by incorporation of tubulin subunits at the kinetochore and, once assembled, they become incorporated into the mitotic spindle. Regardless of how it occurs, the two sister chromatids of each



(a)



(b)

Figure 14.20 Prometaphase. (a) Fluorescence micrograph of a cultured newt lung cell at the early prometaphase stage of mitosis, just after the nuclear envelope has broken. The microtubules of the mitotic spindle are now able to interact with the chromosomes. The mitotic spindle appears green after labeling with a monoclonal antibody against tubulin, whereas the chromosomes appear blue after labeling with a fluorescent dye. (b) Schematic view of some of the successive steps in chromosome-microtubule interactions during prometaphase. In step 1, a kinetochore has made contact with the sidewall of a microtubule and is capable of utilizing kinetochore-bound motors to slide in either direction along the microtubule. In step 2, a chromosome has become attached to the plus end of a microtubule from one spindle pole (an end-on attachment forming a mono-oriented chromosome). In step 3, the chromosome has become attached in an end-on orientation to microtubules from both poles (forming a bi-oriented chromosome). In step 4, the bi-oriented chromosome has been moved to the center of the cell and will become part of the metaphase plate. Chromosomes at this stage are under tension (as indicated by the space between the chromatids) due to the opposing pulling forces exerted by the microtubules from opposite poles. The chromosome in step 3a has both of its kinetochores attached to microtubules from the same spindle pole. This abnormal syntelic attachment is discussed on page 596. (A: COURTESY OF ALEXEY KHODJAKOV, WADSWORTH CENTER, NY.)

mitotic chromosome ultimately become connected by their kinetochores to microtubules that extend from opposite poles.

Observations in living cells indicate that prometaphase chromosomes associated with spindle microtubules are not moved directly to the center of the spindle but rather oscillate back and forth in both a poleward and antipoleward direction. Ultimately, the chromosomes of a prometaphase cell are moved by a process called **congression** toward the center of the mitotic spindle, midway between the poles (step 4, Figure 14.20b). The forces required for chromosome movements during prometaphase are generated by motor proteins associated with both the kinetochores and arms of the chromosomes (depicted in Figure 14.33a and discussed in the legend). Figure 14.21 shows the consequences of a deficiency of a chromosomal motor protein whose activity pushes chromosomes away from the poles.

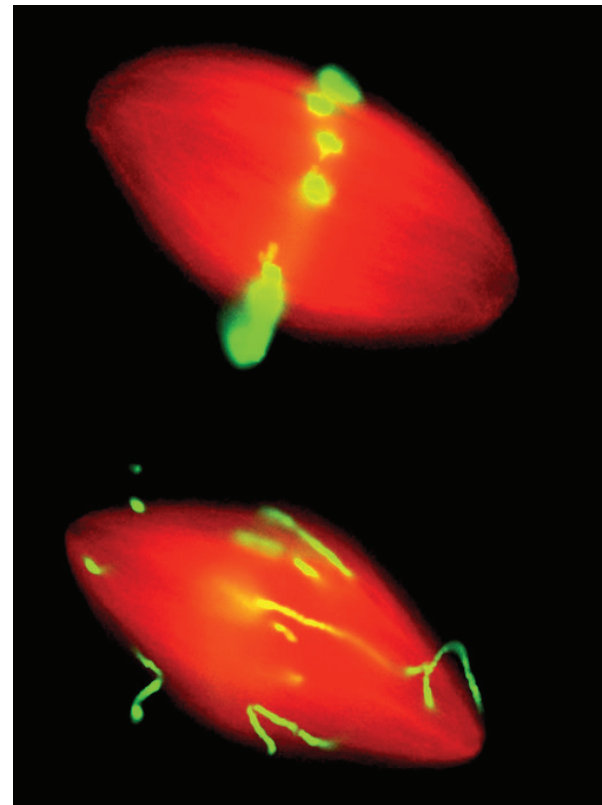
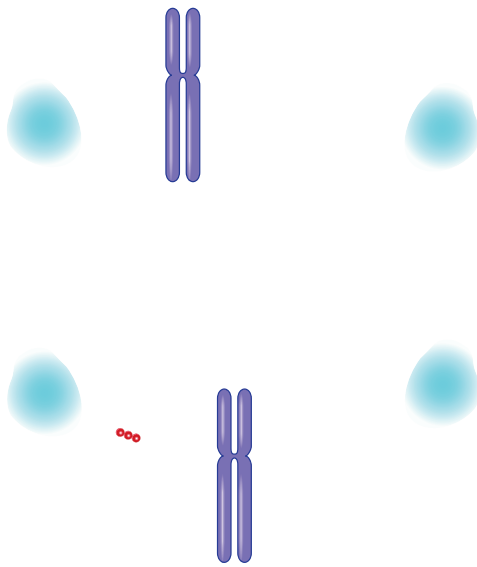


Figure 14.21 The consequence of a missing motor protein on chromosome alignment during prometaphase. The top micrograph shows a mitotic spindle that has assembled in a complete frog egg extract. The lower micrograph shows a mitotic spindle that has assembled in a frog egg extract that has been depleted of a particular kinesin-related protein called Kid that is present along the arms of prometaphase chromosomes. In the absence of this motor protein, the chromosomes fail to align at the center of the spindle and instead are found stretched along spindle microtubules and clustered near the poles. Kid normally provides force for moving chromosomes away from the poles (see Figure 14.33a). (FROM CELIA ANTONIO ET AL., CELL VOL. 102, COVER #4, 2000; WITH PERMISSION FROM ELSEVIER. COURTESY OF ISABELLE VERNOS.)



Microtubule dynamics also play a key role in facilitating chromosome movements during prometaphase. As the chromosomes congress toward the center of the mitotic spindle, the longer microtubules attached to one kinetochore are shortened, while the shorter microtubules attached to the sister kinetochore are elongated. These changes in microtubule length are thought to be governed by differences in pulling force (tension) on the two sister kinetochores. Shortening and elongation of microtubules occur primarily by loss or gain of subunits at the plus end of the microtubule (Figure 14.22). Remarkably, this dynamic activity occurs while the plus end of each microtubule remains attached to a kinetochore.

Eventually, each chromosome moves into position along a plane at the center of the spindle, so that microtubules from each pole are equivalent in length. The movement of a wayward chromosome from a peripheral site near one of the poles to the center of the mitotic spindle during prometaphase is shown in the series of photos in Figure 14.23.

Metaphase

Once all of the chromosomes have become aligned at the spindle equator—with one chromatid of each chromosome

connected by its kinetochore to microtubules from one pole and its sister chromatid connected by its kinetochore to microtubules from the opposite pole—the cell has reached the stage of **metaphase** (Figure 14.24). The plane of alignment of the chromosomes at metaphase is referred to as the *metaphase plate*. The mitotic spindle of the metaphase cell contains a highly organized array of microtubules that is ideally suited for the task of separating the duplicated chromatids positioned at the center of the cell. Functionally and spatially, the microtubules of the metaphase spindle of an animal cell can be divided into three groups (Figure 14.24):

1. *Astral microtubules* that radiate outward from the centrosome into the region outside the body of the spindle. They help position the spindle apparatus in the cell and may help determine the plane of cytokinesis.
2. *Chromosomal (or kinetochore) microtubules* that extend between the centrosome and the kinetochores of the chromosomes. In mammalian cells, each kinetochore is attached to a bundle of 20–30 microtubules, which forms a spindle *fiber* (or *k-fiber*). During metaphase, the chromosomal microtubules exert a pulling force on the kinetochores. As a result, the chromosomes are maintained in the equatorial plane by a “tug-of-war” between balanced pulling forces exerted by chromosomal spindle fibers from opposite poles. These pulling forces generate deformations within the kinetochore and cause oscillations of the chromosomes situated at the metaphase plate. During anaphase, chromosomal microtubules are required for the movement of the chromosomes toward the poles.
3. *Polar (or interpolar) microtubules* that extend from the centrosome past the chromosomes. Polar microtubules from one centrosome overlap with their counterparts from the opposite centrosome. The polar microtubules form a structural basket that maintains the mechanical integrity of the spindle.

As one watches films or videos of mitosis, metaphase appears as a stage during which the cell pauses for a brief period, as if all mitotic activities suddenly come to a halt. However, experimental analysis reveals that metaphase is a time when important events occur.

Microtubule Flux in the Metaphase Spindle Even though there is no obvious change in length of the chromosomal microtubules as the chromosomes are aligned at the metaphase plate, studies using fluorescently labeled tubulin indicate that the microtubules exist in a highly dynamic state. Subunits are rapidly lost and added at the plus ends of the chromosomal microtubules, even though these ends are attached to the kinetochore. Thus, the kinetochore does not act like a cap at the end of the microtubule, blocking the entry or exit of terminal subunits, but rather it is the site of dynamic activity. Because more subunits are added to the plus end than are lost, there is a net addition of subunits at the kinetochore. Meanwhile, the minus ends of the microtubules experience a net loss, and thus subunits move along the chromosomal microtubules from the kinetochore toward the pole. This *poleward flux* of tubulin

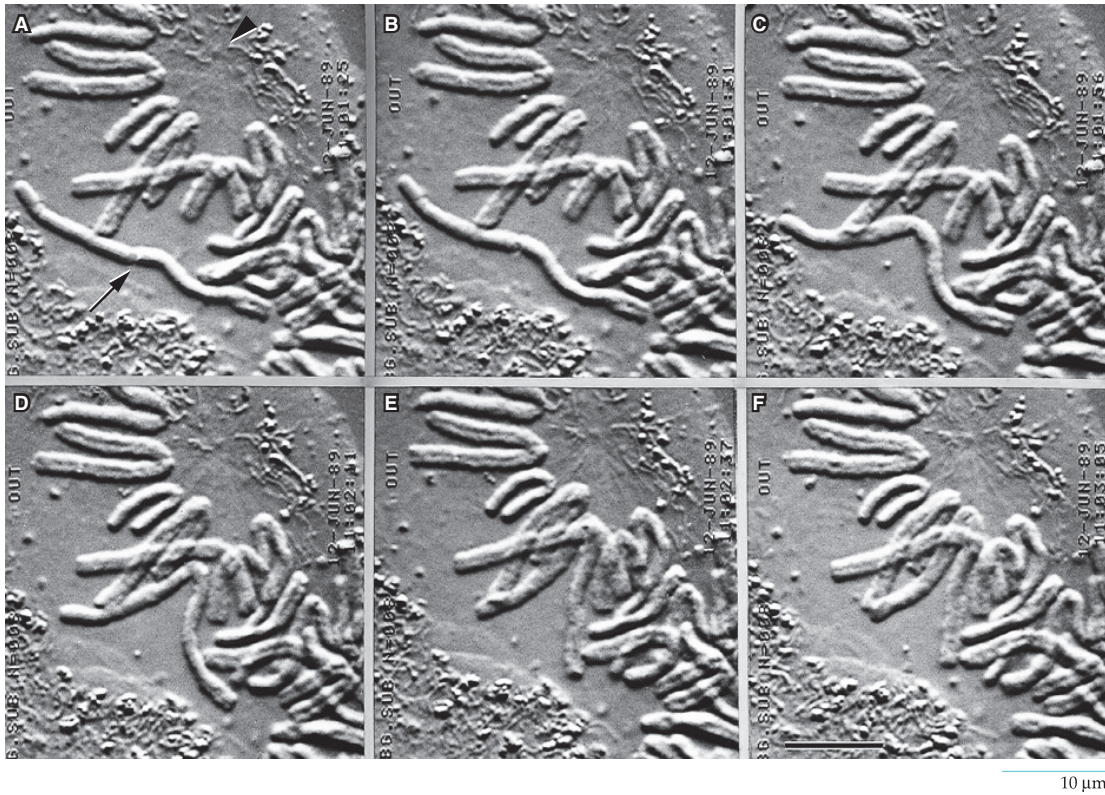


Figure 14.23 The engagement of a chromosome during prometaphase and its movement to the metaphase plate. This series of photographs taken from a video recording shows the movements of the chromosomes of a newt lung cell over a period of 100 seconds during prometaphase. Although most of the cell's chromosomes were nearly aligned at the metaphase plate at the beginning of the sequence, one of the chromosomes (arrow) had failed to become attached to spindle fibers

from both poles. The wayward chromosome has become attached to spindle fibers from opposite poles in B and then moves toward the spindle equator with variable velocity until it reaches a stable position in F. The position of one pole is indicated by the arrowhead in A. (FROM STEPHEN P. ALEXANDER AND CONLY L. RIEDER, *J. CELL BIOL.* 113:807, 1991 FIG. 1. REPRODUCED WITH PERMISSION OF THE ROCKEFELLER UNIVERSITY PRESS. COURTESY CONLY L. RIEDER.)

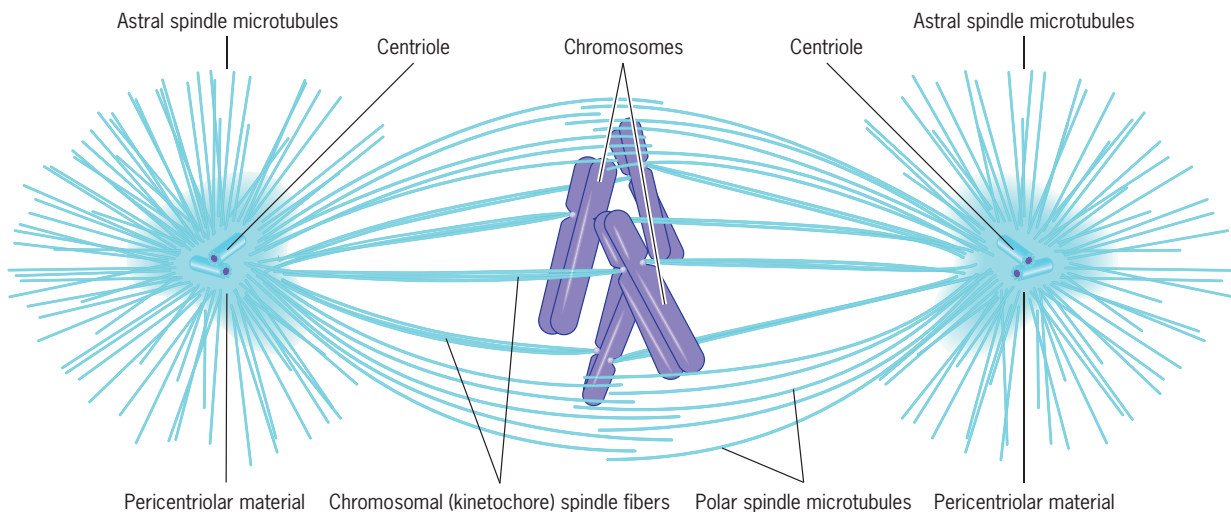


Figure 14.24 The mitotic spindle of an animal cell. Each spindle pole contains a pair of centrioles surrounded by amorphous pericentriolar material at which the microtubules are nucleated. Three types of spindle microtubules—astral, chromosomal, and polar spindle microtubules—are evident, and their functions are described in the

text. All of the spindle microtubules, which can number in the thousands, have their minus ends pointed toward the centrosome. Although not shown here, spindles may also contain shorter microtubules that do not make contact with either a kinetochore or a spindle pole.

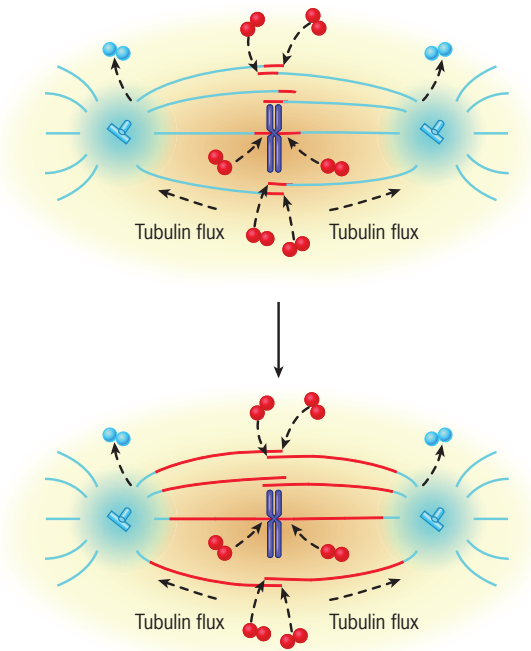


Figure 14.25 Tubulin flux through the microtubules of the mitotic spindle at metaphase. Even though the microtubules appear stationary at this stage, injection of fluorescently labeled tubulin subunits indicates that the components of the spindle are in a dynamic state of flux. Subunits are incorporated preferentially at the kinetochores of the chromosomal microtubules and the equatorial ends of the polar microtubules, and they are lost preferentially from the minus ends of the microtubules in the region of the poles. Tubulin subunits move through the microtubules of a metaphase spindle at a rate of about $1\ \mu\text{m}/\text{min}$.

subunits in a mitotic spindle is indicated in the experiment illustrated in Figure 14.25. Loss of tubulin subunits at the poles is likely aided by a member of the kinesin-13 family of motor proteins whose function is to promote microtubule depolymerization rather than movement (page 336).

Anaphase

Anaphase begins when the sister chromatids of each chromosome split apart and start their movement toward opposite poles.

The Role of Proteolysis in Progression Through Mitosis

We have seen how important it is that specific activities take place in their proper order throughout the cell cycle. This orderliness depends to a large degree on the selective destruction of cell cycle regulatory proteins at precise times during the cell cycle. It was pointed out earlier that two distinct multiprotein complexes, SCF and APC, add ubiquitin to proteins at different stages of the cell cycle, targeting them for destruction by a proteasome. The periods during the cell cycle in which the SCF and APC complexes are active are shown in Figure 14.26a. As illustrated in Figure 14.26a, SCF acts primarily during interphase. In contrast, the *anaphase promoting com-*

plex, or APC, plays a key role in regulating events that occur during mitosis. The APC contains about a dozen core subunits, in addition to an “adaptor protein” that plays a key role in determining which proteins serve as the APC substrate. Two alternate versions of this adaptor protein—Cdc20 and Cdh1—determine substrate selection during mitosis. APC complexes containing one or the other of these adaptors are known as APC^{Cdc20} or APC^{Cdh1} (Figure 14.26a).

APC^{Cdc20} becomes activated prior to metaphase (Figure 14.26a) and ubiquitinates a key anaphase inhibitor called *securin*—so named because it secures the attachment between sister chromatids. The ubiquitination and destruction of securin at the end of metaphase release an active protease called *separase*. Separase then cleaves the Scc1 subunit of the cohesin molecule that holds sister chromatids together (Figure 14.26b). Cleavage of cohesin triggers the separation of sister chromatids to mark the onset of anaphase. Experimental support for the role of cohesin in maintaining the attachment of sister chromatids comes from studies in which proteolytic enzymes have been injected into cells that had been arrested in metaphase. Cleavage of cohesin by such enzymes leads rapidly to the separation of chromatids and their anaphase-like movement towards the poles.

Near the end of mitosis, Cdc20 is inactivated, and the alternate adaptor, Cdh1, takes control of the APC’s substrate selection (Figure 14.26a). When Cdh1 is associated with the APC, the enzyme completes the ubiquitination of cyclin B that was begun by APC^{Cdc20}. Destruction of the cyclin leads to a precipitous drop in activity of the mitotic Cdk (cyclin B–Cdk1) and progression of the cell out of mitosis and into the G₁ phase of the next cell cycle. The importance of protein degradation in regulating the events of mitosis and the reentry of cells into G₁ is best revealed with the use of inhibitors (Figure 14.27). If the destruction of cyclin B is prevented with an inhibitor of the proteasome, cells remain arrested in a late stage of mitosis. If such cells that are arrested in mitosis are subsequently treated with a compound that inhibits the kinase activity of Cdk1, the cell will return to its normal activities and continue through mitosis and cytokinesis. The completion of mitosis clearly requires the cessation of activity of Cdk1 (either by the normal destruction of its cyclin activator or by experimental inhibition). Perhaps the most striking finding of all is obtained when the proteasome-inhibited and Cdk1-inhibited cells that have already exited mitosis are washed free of the Cdk1 inhibitor (Figure 14.27). Such cells, which now contain both cyclin B and active Cdk1, actually progress in the reverse direction and head back into mitosis. This reversal is characterized by compaction of the chromosomes, breakdown of the nuclear envelope, assembly of a mitotic spindle, and movement of the chromosomes back to the metaphase plate, as shown in Figure 14.27. All of these events are triggered by the inappropriate reactivation of Cdk1 activity by removal of its inhibitor. This finding dramatically illustrates the importance of proteolysis in moving the normal cell cycle in a single, irreversible direction.

The Events of Anaphase All the chromosomes of the metaphase plate are split in synchrony at the onset of anaphase, and the chromatids (now referred to as chromosomes,

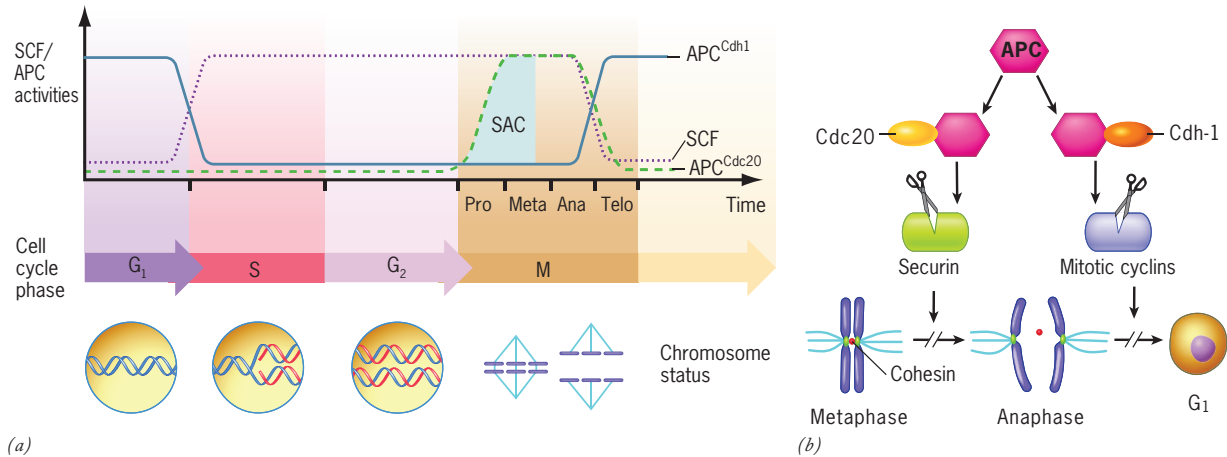


Figure 14.26 SCF and APC activities during the cell cycle. SCF and APC are multisubunit complexes that ubiquitinate substrates, leading to their destruction by proteasomes. (a) SCF is active primarily during interphase, whereas APC (anaphase promoting complex) is active during mitosis and G₁. Two different versions of APC are indicated. These two APCs differ in containing either a Cdc20 or a Cdh1 adaptor protein, which alters the substrates recognized by the APC. APC^{Cdc20} is active early in mitosis, at a time when Cdh1 is inhibited by Cdk1-mediated phosphorylation. As Cdk1 activity drops sharply in late mitosis, Cdh1 is activated, leading to the activation of APC^{Cdh1}. The label SAC stands for spindle assembly checkpoint, which is discussed on page 596. The SAC prevents APC^{Cdc20} from triggering anaphase until all the chromosomes are properly aligned at the metaphase plate. (b) APC^{Cdc20} is responsible for destroying proteins, such as securin, that inhibit anaphase. Destruction of these

substrates promotes the metaphase–anaphase transition. APC^{Cdh1} is responsible for ubiquitinating proteins, such as mitotic cyclins, that inhibit exit from mitosis. Destruction of these substrates promotes the mitosis–G₁ transition. APC^{Cdh1} activity during early G₁ helps maintain the low cyclin–Cdk activity (Figure 14.8) required to assemble prereplication complexes at the origins of replication (Figure 13.20). (Although not discussed here, the activation of phosphatases that remove the phosphate groups added by Cdk1 also plays a substantial part in driving the events that occur during the latter stages of mitosis and reentry into G₁; see *Nature Revs. Mol. Cell Biol.* 12:469, 2011.) (A: AFTER J-M PETERS, *CURR. OPIN. CELL BIOL.* 10:762, 1998; COPYRIGHT 1998. CURRENT OPINION IN CELL BIOLOGY BY ELSEVIER LTD. REPRODUCED WITH PERMISSION OF ELSEVIER LTD. IN THE FORMAT REUSE IN A BOOK/TEXTBOOK VIA COPYRIGHT CLEARANCE CENTER. SEE ALSO *NATURE REVs. MOL. CELL BIOL.* 7:650, 2006.)

because they are no longer attached to their sisters) begin their poleward migration (see Figure 14.11). As each chromosome moves during anaphase, its centromere is seen at its leading

edge with the arms of the chromosome trailing behind (Figure 14.28a). The movement of chromosomes toward opposite poles is very slow relative to other types of cellular movements,

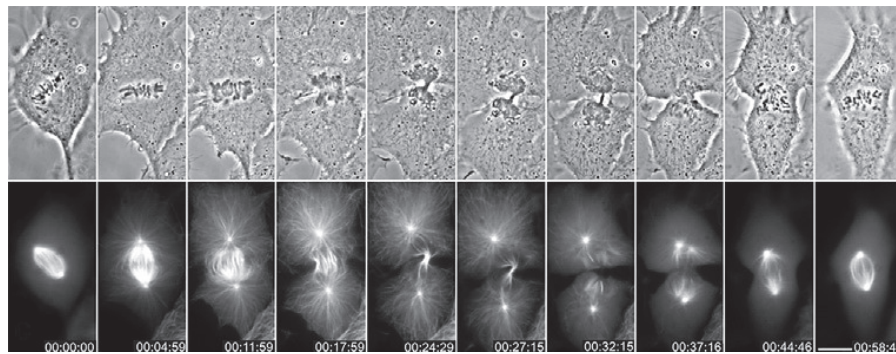
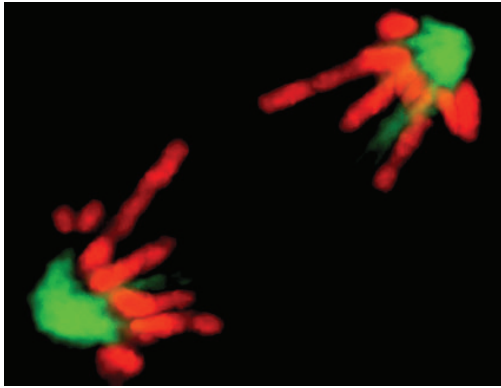


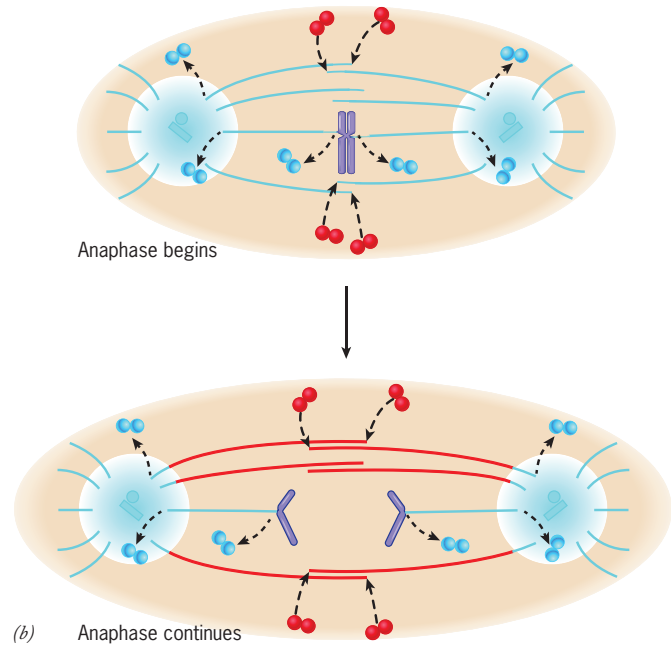
Figure 14.27 Experimental demonstration of the importance of proteolysis in a cell's irreversible exit from mitosis. This illustration shows frames from a video of a cell that had been arrested in mitosis by the presence of a proteasome inhibitor (MG132). At time 0, a Cdk1 inhibitor (flavopiridol) was added to the medium, which caused the cell to complete mitosis and initiate cytokinesis. At 25 min, the cell was washed free of the Cdk1 inhibitor. Because the cell still contained cyclin B (which would normally have been destroyed by proteasomes), the cell reentered mitosis and progressed to metaphase as seen in the

last five frames of the video. The upper row shows phase-contrast images of the cell at various times, and the lower row shows the corresponding fluorescence micrographs with times indicated. Video 3, from which these frames were taken, can be seen at the online version of this paper. Bar in lower right image equals 10 μ m. (FROM TAMARA A. POTAPOVA, ET AL., *NATURE* 440:954, 2006. © 2006, REPRINTED WITH PERMISSION FROM MACMILLAN PUBLISHERS LIMITED. COURTESY OF GARY J. GORBSKY.)



(a)

Figure 14.28 The mitotic spindle and chromosomes at anaphase. (a) Fluorescence micrograph of a cell at late anaphase, showing the highly compacted arms of the chromosomes at this stage. The arms are seen to trail behind as the kinetochores lead the way toward the respective poles. The chromosomal spindle fibers at this late anaphase stage are extremely short and are no longer evident between the forward edges of the chromosomes and the poles. The polar spindle microtubules, however, are clearly visible in the interzone between the separating chromosomes. Relative movements of the polar microtubules are thought to be responsible for causing the separation of the poles that occurs during anaphase B. (b) Microtubule dynamics during anaphase. Tubulin subunits are lost from both ends of the chromosomal microtubules, resulting in shortening of chromosomal fibers and movement of the chromosomes toward the poles during anaphase A. Meanwhile, tubulin subunits are added to the plus ends of polar



microtubules, which also slide across one another, leading to separation of the poles during anaphase B. (A: FROM FELIPE MORA-BERMÚDEZ, DANIEL GERLICH, AND JAN ELLENBERG, COVER OF NATURE CELL BIOL. 9, #7, 2007; © 2007, REPRINTED BY PERMISSION FROM MACMILLAN PUBLISHERS LIMITED.)

proceeding at approximately $1\text{ }\mu\text{m}$ per minute, a value calculated by one mitosis researcher to be equivalent to a trip from North Carolina to Italy that would take approximately 14 million years. The slow rate of chromosome movement ensures that the chromosomes segregate accurately and without entanglement. The forces thought to power chromosome movement during anaphase are discussed in the following section.

The poleward movement of chromosomes is accompanied by obvious shortening of chromosomal microtubules. It has long been appreciated that tubulin subunits are lost from the plus (kinetochore-based) ends of chromosomal microtubules during anaphase (Figure 14.28b). Subunits are also lost from the minus ends of these microtubules as a result of the continued poleward flux of tubulin subunits that occurs during prometaphase and metaphase (Figures 14.22 and 14.25). The primary difference in microtubule dynamics between metaphase and anaphase is that subunits are added to the plus ends of microtubules during metaphase, keeping the length of the chromosomal fibers constant (Figure 14.25), whereas subunits are lost from the plus ends during anaphase, resulting in shortening of the chromosomal fibers (Figure 14.28b). This change in behavior at the microtubule plus ends is thought to be triggered by the loss of tension on the kinetochores following separation of the sister chromatids.

The movement of the chromosomes toward the poles is referred to as **anaphase A** to distinguish it from a separate but

simultaneous movement, called **anaphase B**, in which the two spindle poles move farther apart. The elongation of the mitotic spindle during anaphase B is accompanied by the net addition of tubulin subunits to the plus ends of the polar microtubules. Thus, subunits can be preferentially added to polar microtubules and removed from chromosomal microtubules at the same time in different regions of the same mitotic spindle (Figure 14.28b).

Forces Required for Chromosome Movements at Anaphase In the early 1960s, Shinya Inoué of the Marine Biological Laboratory at Woods Hole proposed that the depolymerization of chromosomal microtubules during anaphase was not simply a consequence of chromosome movement but the cause of it. Inoué suggested that depolymerization of the microtubules that comprise a spindle fiber could generate sufficient mechanical force to pull a chromosome forward.

Early experimental support for the disassembly-force model came from studies in which chromosomes underwent considerable movement as the result of the depolymerization of attached microtubules. An example of one of these experiments is shown in Figure 14.29. In this case, the movement of a microtubule-bound chromosome (arrow) occurs *in vitro* following dilution of the medium. Dilution reduces the concentration of soluble tubulin, which in turn promotes the depolymerization of the microtubules. These types of experi-

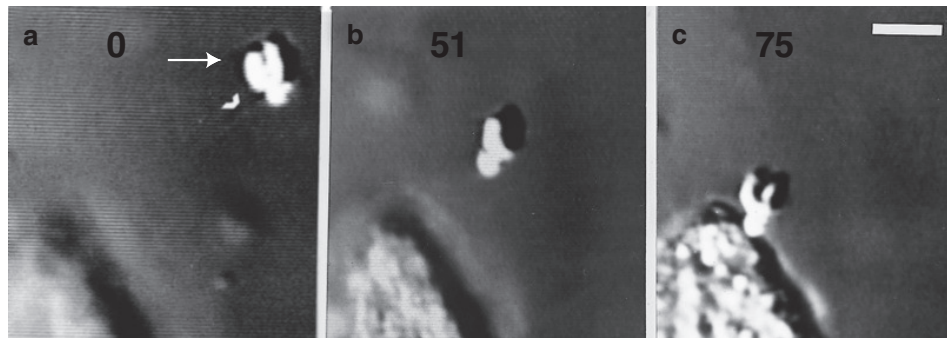


Figure 14.29 Experimental demonstration that microtubule depolymerization can move attached chromosomes *in vitro*. The structure at the lower left is the remnant of a lysed protozoan. In the presence of tubulin, the basal bodies at the surface of the protozoan were used as sites for the initiation of microtubules, which grew outward into the medium. Once the microtubules had formed, condensed mitotic chromosomes were introduced into the chamber and allowed to bind to the ends of the microtubules. The arrow shows a

chromosome attached to the end of a bundle of microtubules. The concentration of soluble tubulin within the chamber was then decreased by dilution, causing the depolymerization of the microtubules. As shown in this video sequence, the shrinkage of the microtubules was accompanied by the movement of the attached chromosome. Bar equals 5 μm . (FROM MARTINE COUE, VIVIAN A. LOMBILLO, AND J. RICHARD MCINTOSH, *J. CELL BIOL.* 112:1169, 1991, FIG. 3. REPRODUCED WITH PERMISSION OF THE ROCKEFELLER UNIVERSITY PRESS.)

ments, as well as direct force-measuring studies, indicate that microtubule depolymerization alone can generate sufficient force to pull chromosomes through a cell.

Figure 14.30*a* depicts a model of the events proposed to occur during chromosome movement at anaphase in a vertebrate cell. As indicated in Figure 14.28*b*, the microtubules that comprise the chromosomal spindle fibers undergo depolymerization at both their minus and plus ends during anaphase. These combined activities lead to the movement of chromosomes toward the pole. Depolymerization at the microtubule minus ends serves to transport the chromosomes

toward the poles due to poleward flux, reminiscent of a person standing on a “moving walkway” in an airport. In contrast, depolymerization at the microtubule plus ends serves to “chew up” the fiber that is towing the chromosomes. Some cells rely more on poleward flux, others more on plus-end depolymerization. Studies of animal cells in anaphase have revealed that both the plus and minus ends of chromosomal fibers are sites where depolymerizing kinesins (members of the kinesin-13 family, page 336) are localized. These depolymerases are indicated at the opposite ends of the microtubule in Figure 14.30*a*. If either of these microtubule “depolymerases” is specifically

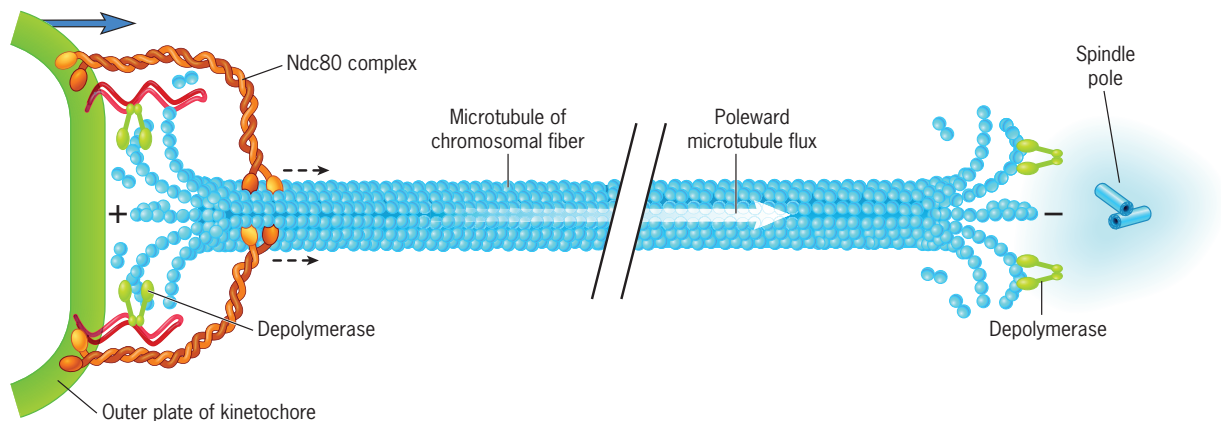


Figure 14.30 Proposed mechanism for the movement of chromosomes during anaphase in animal cells. In the model depicted here, chromosome movement toward the poles is accomplished by a combination of poleward flux, which moves the body of each microtubule toward one of the poles, and simultaneous depolymerization of the microtubule at both ends. Depolymerizing kinesins of the kinesin-13 family have been localized at both the plus (kinetochore) and minus (polar) ends of chromosomal microtubules and are postulated to be responsible for depolymerization at their respective sites. In this model, the Ndc80 protein complex of the outer

kinetochore plate acts as the device that couples microtubule depolymerization to chromosome segregation. The force required for chromosome movement is provided by the release of strain energy as the microtubule depolymerizes. The released energy is utilized by the curled ends of the depolymerizing protofilaments to bias the movement of the bound heads of the Ndc80 complex (dashed black arrow) toward the minus end of the microtubule. Motor proteins of the kinetochore, such as cytoplasmic dynein, may also have a force-generating role in chromosome movement during anaphase.

inhibited, chromosome segregation during anaphase is at least partially disrupted. These findings suggest that ATP-dependent, kinesin-mediated depolymerization forms the basis for chromosome segregation during mitosis.

It was mentioned on page 585 that one of the questions of greatest interest in the field of mitosis is the mechanism by which kinetochores are able to hold on to the plus ends of microtubules that are losing tubulin subunits. The Ndc80 complexes of the kinetochore are present as molecular fibrils that reach out to form relatively weak linkages with an attached microtubule just behind its plus end. It is estimated that each microtubule is contacted around its circumference by 6 to 9 of these Ndc80 tethers. A number of studies suggest that, as indicated in Figure 14.30, the terminal heads of the Ndc80 complexes are able to travel along the microtubule towards its minus end, pushed along by the curling protofilaments of the disassembling tip. As a result, the attached chromosome moves toward the spindle pole as it is towed by the shrinking chromosomal fiber.

The Spindle Assembly Checkpoint As discussed on page 579, cells possess checkpoint mechanisms that monitor the status of events during the cell cycle. One of these checkpoints operates at the transition between metaphase and anaphase. The **spindle assembly checkpoint (SAC)**, as it is called, is best revealed when a chromosome fails to become aligned properly at the metaphase plate. When this happens, the checkpoint mechanism delays the onset of anaphase until the misplaced chromosome has assumed its proper position along the spindle equator. If a cell were not able to postpone chromosome segregation, it would greatly elevate the risk of the daughter cells receiving an abnormal number of chromosomes (aneuploidy). This expectation has been confirmed with the identification of a number of children with inherited deficiencies in one of the spindle checkpoint proteins. These individuals exhibit a disorder (named MVA), which is characterized by a high percentage of aneuploid cells and a greatly increased risk of developing cancer.

How does the cell determine whether or not all of the chromosomes are properly aligned at the metaphase plate? Let's consider a chromosome that is only attached to microtubules from one spindle pole, which is probably the circumstance of the wayward chromosome indicated by the arrow in Figure 14.23a. Unattached kinetochores contain a complex of proteins, the best studied of which is called Mad2, that mediates the spindle assembly checkpoint. The presence of these proteins at an *unattached* kinetochore sends a "wait" signal to the cell cycle machinery that prevents the cell from continuing on into anaphase. Once the wayward chromosome becomes attached to spindle fibers from both spindle poles and becomes properly aligned at the metaphase plate, the signaling complex leaves the kinetochore, which turns off the "wait" signal and allows the cell to progress into anaphase.

Figure 14.31 shows the mitotic spindle of a cell that is arrested prior to metaphase due to a single unaligned chromosome. Unlike all of the other kinetochores in this cell, only the unaligned chromosome is seen to still contain the Mad2 protein. As long as the cell contains unaligned chromosomes,

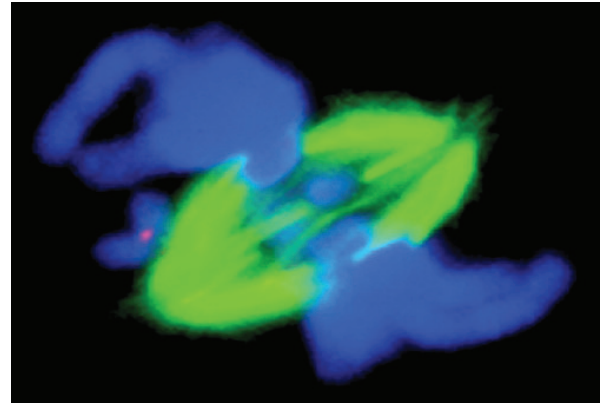


Figure 14.31 The spindle assembly checkpoint. Fluorescence micrograph of a mammalian cell in late prometaphase labeled with antibodies against the spindle checkpoint protein Mad2 (pink) and tubulin of the microtubules (green). The chromosomes appear blue. Only one of the chromosomes of this cell is seen to contain Mad2, and this chromosome has not yet become aligned at the metaphase plate. The presence of Mad2 on the kinetochore of this chromosome is sufficient to prevent the initiation of anaphase. (FROM JENNIFER WATERS, REY-HUEI CHEN, ANDREW W. MURRAY, AND E. D. SALMON, *J. CELL BIOL.* 141, COVER #5, 1998; REPRODUCED WITH PERMISSION OF THE ROCKEFELLER UNIVERSITY PRESS.)

Mad2 molecules are able to inhibit cell cycle progress. According to a favored model, inhibition is achieved through direct interaction between Mad2 and the APC activator Cdc20. During the period that Cdc20 is bound to Mad2, APC complexes would be unable to ubiquitinate the anaphase inhibitor securin, thus keeping all of the sister chromatids attached to one another by their cohesin "glue."

It is well established that the spindle assembly checkpoint is activated by the presence of an unattached kinetochore, but there are other chromosomal abnormalities that arise during the progression to metaphase that also require corrective measures. For example, on occasion, the two kinetochores of sister chromatids will become attached to microtubules from the same spindle pole, a condition referred to as a syntelic attachment (step 3a, Figure 14.20b). If not corrected, a syntelic attachment is very likely to lead to the movement of both sister chromatids to one of the daughter cells, leaving the other daughter devoid of this chromosome. The cell is likely alerted to the presence of a chromosome with a syntelic attachment by the lack of tension on the chromosome's kinetochores. Tension normally develops when sister chromatids are being pulled by microtubules from opposite spindle poles (steps 3–4, Figure 14.20b).

Cells are able to correct syntelic attachments (and other types of abnormal microtubule connections) through the action of an enzyme called Aurora B kinase, which is part of a mobile protein complex that resides at the centromere during prometaphase and metaphase. Among the substrates of Aurora B kinase are several of the proteins thought to be involved in kinetochore–microtubule attachment, including members of the Ndc80 complex and the kinesin depolymerase of Figure

14.30. Studies suggest that Aurora B kinase molecules of an incorrectly attached chromosome phosphorylate these protein substrates, which destabilizes microtubule attachment to both kinetochores. Once freed of their bonds, the kinetochores of each sister chromatid have a fresh opportunity to become attached to microtubules from opposite spindle poles. Inhibition of Aurora B kinase in cells or extracts leads to misalignment and missegregation of chromosomes (see Figure 18.51).

Telophase

As the chromosomes near their respective poles, they tend to collect in a mass, which marks the beginning of the final stage of mitosis, or **telophase** (Figures 14.11 and 14.32). During telophase, daughter cells return to the interphase condition: the mitotic spindle disassembles, the nuclear envelope reforms, and the chromosomes become more and more dispersed until they disappear from view under the microscope. The actual partitioning of the cytoplasm into two daughter cells occurs by a process to be discussed shortly. First, however,

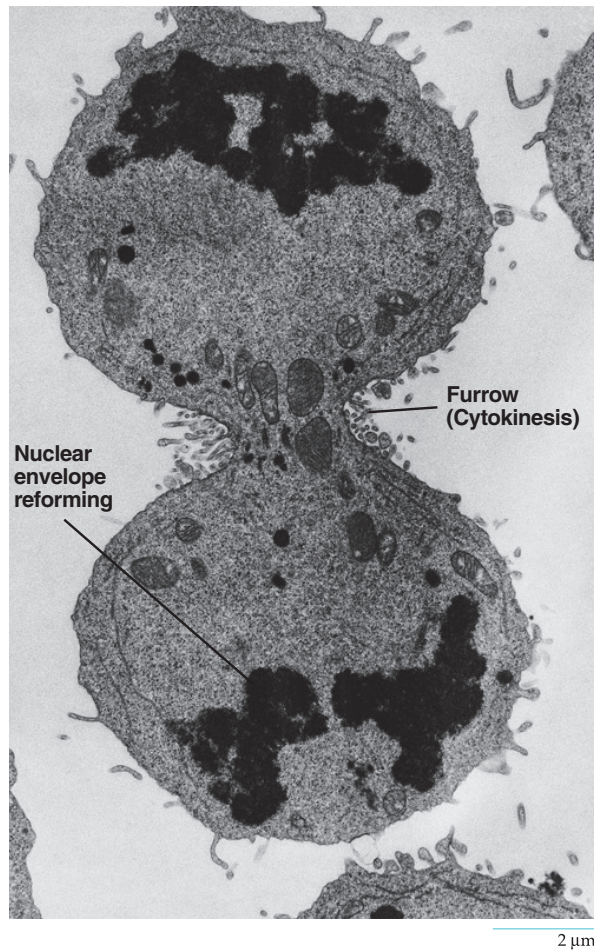


Figure 14.32 Telophase. Electron micrograph of a section through a human lymphocyte in telophase. (DAVID M. PHILLIPS/PHOTO RESEARCHERS, INC.)

let's look back and consider the motor proteins involved in several of the major chromosome movements that take place during mitosis.

Motor Proteins Required for Mitotic Movements

Mitosis is characterized by extensive movements of cellular structures. Prophase is accompanied by movement of the spindle poles to opposite ends of the cell, prometaphase by movement of the chromosomes to the spindle equator, anaphase A by movement of the chromosomes from the spindle equator to its poles, and anaphase B by the elongation of the spindle. Over the past decade, a variety of different molecular motors have been identified in different locations in mitotic cells of widely diverse species. The motors involved in mitotic movements are primarily microtubule motors, including a number of different kinesin-related proteins and cytoplasmic dynein. Some of the motors move toward the plus end of the microtubule, others toward the minus end. As discussed above, one group of kinesins does not move anywhere, but promotes microtubule depolymerization. Motor proteins have been localized at the spindle poles, along the spindle fibers, and within both the kinetochores and arms of the chromosomes. Although firm conclusions about the functions of specific motor proteins cannot yet be drawn, a general picture of the roles of these molecules is suggested (Figure 14.33):

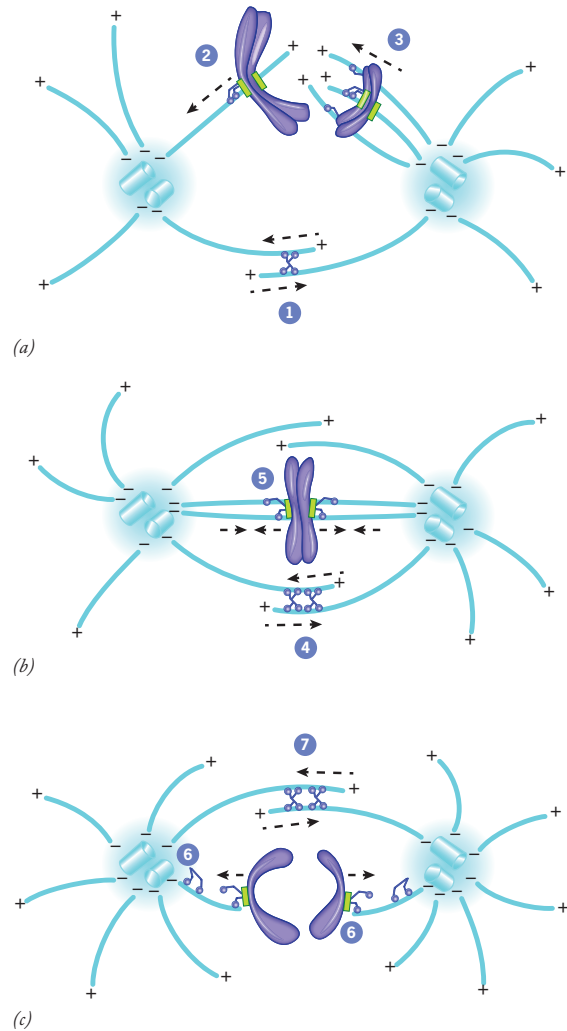
- Motor proteins located along the polar microtubules probably contribute by keeping the poles apart (Figure 14.33*a,b*).
- Motor proteins residing on the chromosomes are probably important in the movements of the chromosomes during prometaphase (Figure 14.33*a*), in maintaining the chromosomes at the metaphase plate (Figure 14.33*b*), and in separating the chromosomes during anaphase (Figure 14.33*c*).
- Motor proteins situated along the overlapping polar microtubules in the region of the spindle equator are probably responsible for cross-linking antiparallel microtubules and sliding them over one another, thus elongating the spindle during anaphase B (Figure 14.33*c*).

Cytokinesis

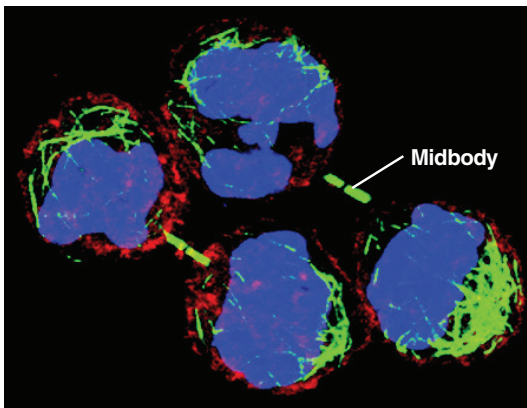
Mitosis accomplishes the segregation of duplicated chromosomes into daughter nuclei, but the cell is divided into two daughter cells by a separate process called **cytokinesis**. The first hint of cytokinesis in most animal cells appears during anaphase as an indentation of the cell surface in a narrow band around the cell. As time progresses, the indentation deepens to form a furrow that moves inward toward the center of the cell. The plane of the furrow lies in the same plane previously occupied by the chromosomes of the metaphase plate, so that the two sets of chromosomes are ultimately partitioned into different cells (as in Figure 14.32). As one cell becomes two cells, additional plasma membrane is delivered to the cell surface via cytoplasmic vesicles that fuse with the advancing

Figure 14.33 Proposed activity of motor proteins during mitosis.

(a) Prometaphase. The two halves of the mitotic spindle are moving apart from one another to opposite poles, due to the action of bipolar (4-headed) plus-end-directed motors (members of the kinesin-5 family). These motors can bind by their heads to antiparallel microtubules from opposite poles and cause them to slide apart (step 1). (Additional motors associated with the centrosomes and cortex are not shown.) Meanwhile, the chromosomes have become attached to the chromosomal microtubules and can be seen oscillating back and forth along the microtubules. Ultimately, the chromosomes are moved to the center of the spindle, midway between the poles. Poleward chromosome movements are mediated by minus-end-directed motors (i.e., cytoplasmic dynein) residing at the kinetochore (step 2). Chromosome movements away from the poles are mediated by plus-end-directed motors (i.e., kinesins) residing at the kinetochore and especially along the chromosome arms (step 3) (see Figure 14.21). (b) Metaphase. The two halves of the spindle maintain their separation as the result of continued plus-end-directed motor activity associated with the polar microtubules (step 4). The motor activity associated with step 4 is also suspected of promoting the flux of subunits within these microtubules that is depicted in Figure 14.25. The chromosomes are thought to be maintained at the equatorial plane by the balanced activity of plus end- and minus end-directed motor proteins residing at the kinetochore (step 5). (c) Anaphase. The movement of the chromosomes toward the poles is thought to require the activity of kinesin depolymerases that catalyze depolymerization at both the plus and minus ends of microtubules (step 6). The separation of the poles (anaphase B) is thought to result from the continuing activity of the bipolar plus-end-directed motors of the polar microtubules (step 7). (K. E. SAWIN AND J. M. SCHOLEY, TRENDS CELL BIOL. 1:122, 1991. TRENDS IN CELL BIOLOGY BY ELSEVIER LTD. REPRODUCED WITH PERMISSION OF ELSEVIER LTD. IN THE FORMAT REUSE IN A BOOK/TEXTBOOK VIA COPYRIGHT CLEARANCE CENTER.)



cleavage furrow. In its latter stages, the advancing furrow passes through the tightly packed remnants of the central portion of the mitotic spindle, which forms a cytoplasmic bridge between the daughter cells called the *midbody* (Figure 14.34a). The final step of cytokinesis is called *abscission*, when the surfaces of the cleavage furrow fuse with one another, splitting



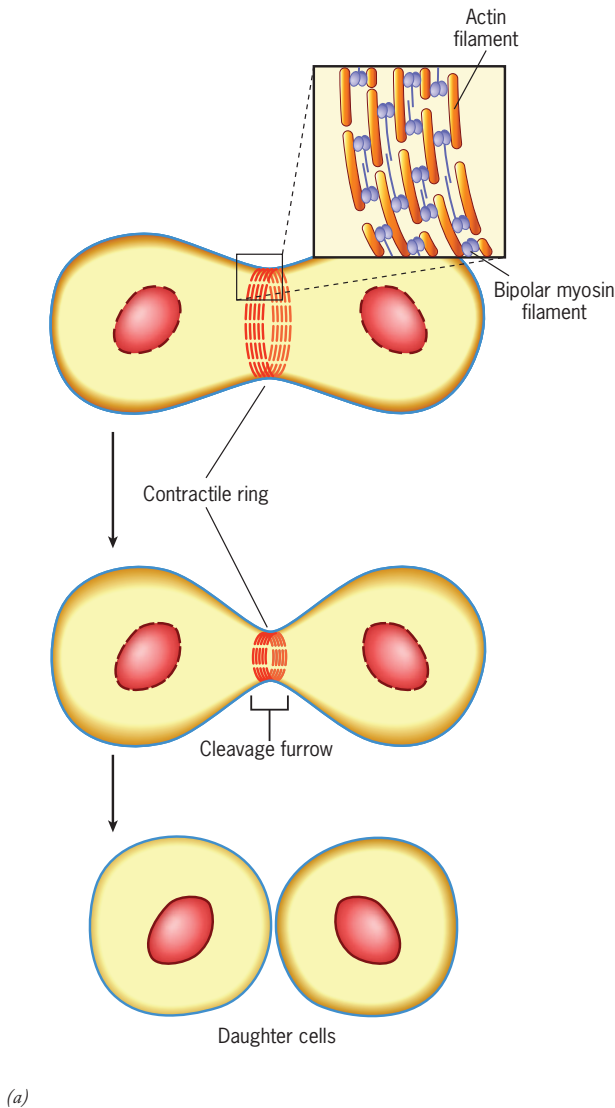
(a)

Figure 14.34 Cytokinesis (a) These cultured mammalian cells are undergoing the final step in cytokinesis, called *abscission*, in which the cleavage furrow cuts through the midbody, a thin cytoplasmic bridge that is packed with remnants of the central portion of the mitotic spindle. Microtubules are green, actin is red, and DNA is blue.



(b)

(b) This fertilized sea urchin egg has just been split into two cells by cytokinesis. (FROM AHINA R. SKOP ET AL., SCIENCE 305:61, 2004, FIG. 1A. REPRINTED WITH PERMISSION FROM AAAS. B: COURTESY OF TRYGGVE GUSTAFSON.)



the cell in two (Figure 14.34*b*). Abscission requires the action of ESCRT complexes—the same proteins responsible for severing the intraluminal vesicles that form within endosomes (page 312).

Our present concept of the mechanism responsible for cytokinesis stems from a proposal made by Douglas Marsland in the 1950s known as the *contractile ring theory* (Figure 14.35*a*). Marsland proposed that the force required to cleave a cell is generated in a thin band of contractile cytoplasm located in the *cortex*, just beneath the plasma membrane of the furrow. Microscopic examination of the cortex beneath the furrow of a cleaving cell reveals the presence of large numbers of actin filaments (Figure 14.35*b* and 14.36*a*). These unbranched actin filaments are assembled in the cell cortex by the action of a formin protein (page 372), which may also anchor the filaments to the overlying plasma membrane.

Interspersed among the actin filaments are short, bipolar myosin filaments. These filaments are composed of myosin II,

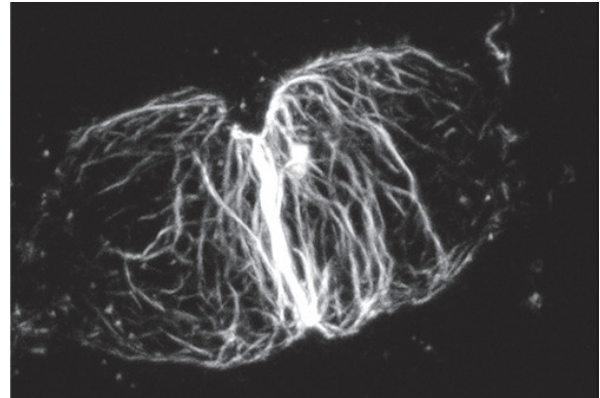


Figure 14.35 The formation and operation of the contractile ring during cytokinesis. (a) Actin filaments become assembled in a ring at the cell equator. Contraction of the ring, which requires the action of myosin, causes the formation of a furrow that splits the cell in two. (b) Confocal fluorescence micrograph of a fly spermatocyte undergoing cytokinesis at the end of the first meiotic division. Actin filaments, which have been stained with the mushroom toxin phalloidin, are seen to be concentrated in a circular equatorial band within the cleavage furrow: (b: FROM DANIEL SAUL, ET AL., J. CELL SCIENCE 117:3893, 2004, #17 COVER, COURTESY OF JULIE A. BRILL, BY PERMISSION OF THE COMPANY OF BIOLOGISTS LTD. <http://jcs.biologists.org/content/117/17.cover-expansion>)

as evidenced by their binding of anti-myosin II antibodies (Figure 14.36*b*). The importance of myosin II in cytokinesis is evident from the fact that (1) anti-myosin II antibodies cause the rapid cessation of cytokinesis when injected into a dividing cell (Figure 14.36*c*), and (2) cells lacking a functional myosin II gene carry out nuclear division by mitosis but cannot divide normally into daughter cells. The assembly of the actin-myosin contractile machinery in the plane of the future cleavage furrow is orchestrated by a G protein called RhoA. In its GTP-bound state, RhoA triggers a cascade of events that leads to both the assembly of actin filaments and the activation of myosin II's motor activity. If RhoA is depleted from cells or inactivated, a cleavage furrow fails to develop.

The force-generating mechanism operating during cytokinesis is thought to be similar to the actin and myosin-based contraction of muscle cells. In fact, the cytokinesis furrow of a primitive single-celled eukaryote is the likely evolutionary ancestor of the contractile machinery of animal muscle cells. Whereas the sliding of actin filaments of a muscle cell brings about the shortening of the muscle fiber, sliding of the filaments of the contractile ring pulls the cortex and attached plasma membrane toward the center of the cell. As a result, the contractile ring constricts the equatorial region of the cell, much like pulling on a purse string narrows the opening of a purse.

It is generally agreed that the position of the cleavage furrow is determined by the position of the anaphase mitotic spindle, which induces the activation of RhoA in a narrow ring within the cortex. However, there has been considerable

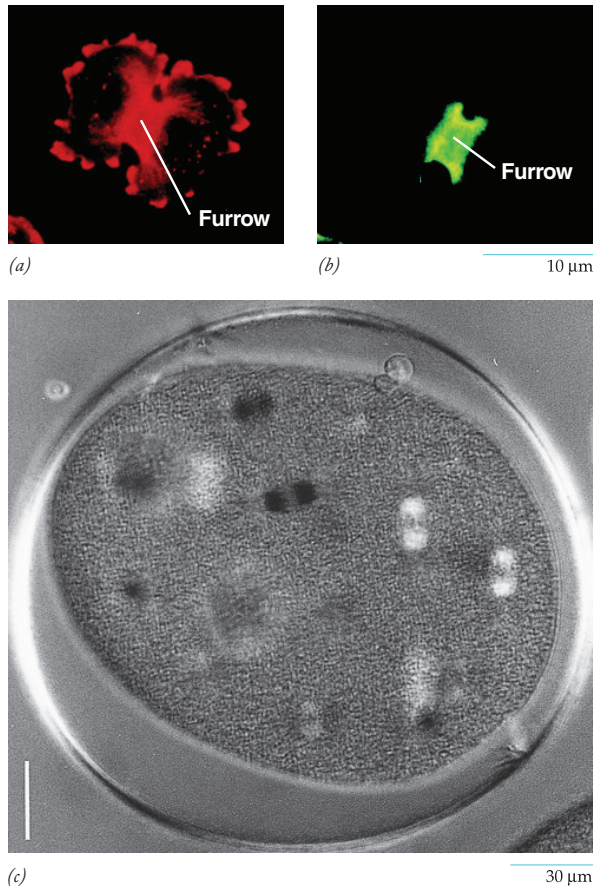


Figure 14.36 Experimental demonstration of the importance of myosin in cytokinesis. (a,b) Localization of actin and myosin II in a *Dictyostelium* ameba during cytokinesis as demonstrated by double-stain immunofluorescence. (a) Actin filaments (red) are located at both the cleavage furrow and the cell periphery where they play a key role in cell movement (Section 9.7). (b) Myosin II (green) is localized at the cleavage furrow, where it is part of a contractile ring that encompasses the equator. (c) A starfish egg that had been microinjected with an antibody against starfish myosin, as observed under polarized light (which causes the mitotic spindles to appear either brighter or darker than the background due to the presence of oriented microtubules). While cytokinesis has been completely suppressed by the antibodies, mitosis (as revealed by the mitotic spindles) continues unaffected. (A,B: COURTESY OF YOSHIO FUKUI; C: FROM DANIEL P. KIEHART, ISSEI MABUCHI, AND SHINYA INOUÉ, J. CELL BIOL. 94:167, 1982, FIG. 1. REPRODUCED WITH PERMISSION OF THE ROCKEFELLER UNIVERSITY PRESS.)

debate as to how this particular zone within the cortex is selected. Early pioneering studies on marine invertebrate eggs by Ray Rappaport of Union College in New York demonstrated that the contractile ring forms in a plane midway between the spindle poles, even if one of the poles is displaced by a microneedle inserted into the cell. An example of the relationship between the position of the spindle poles and cleavage plane is shown in the experiment of Figure 14.37. These studies suggest that the site of actin-filament assembly, and

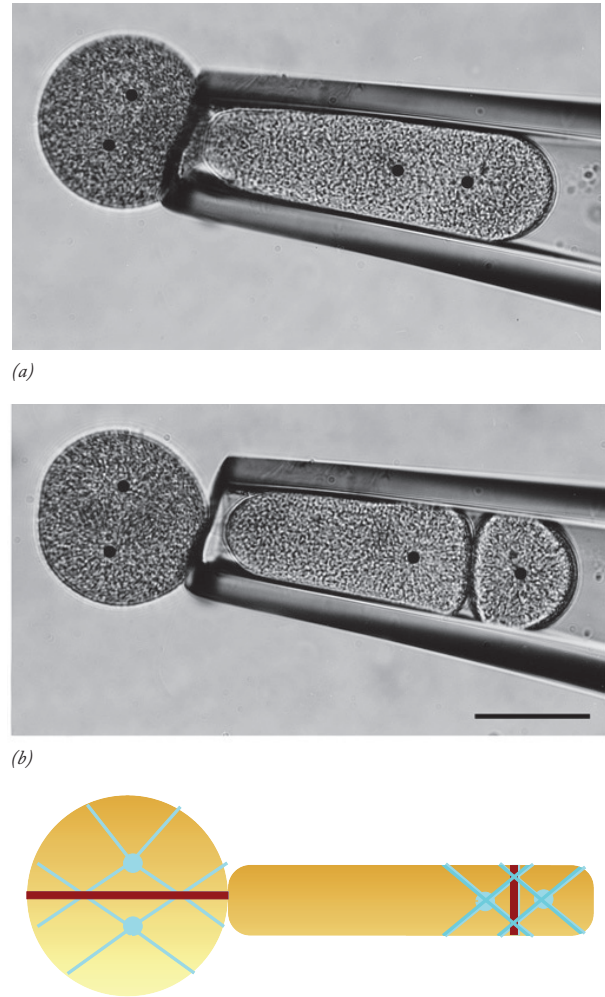


Figure 14.37 The site of formation of the cleavage plane and the time at which cleavage occurs depends on the position of the mitotic spindle. (a) This echinoderm egg was allowed to divide once to form a two-cell embryo. Then, once the mitotic spindle appeared in each of the two cells, one of the cells was drawn into a micropipette, causing it to assume a cylindrical shape. The two dark spots in each cell are the spindle poles that have formed prior to the second division of each cell. (b) Nine minutes later the cylindrical cell has completed cleavage, while the spherical cell has not yet begun to cleave. These photos indicate that (1) the cleavage plane forms between the spindle poles, regardless of their position, and (2) cleavage occurs more rapidly in the cylindrical cell. Bar equals 80 μm . (c) These results can be explained by assuming that (1) the cleavage plane (brown bar) forms where the astral microtubules overlap, and (2) cleavage occurs earlier in the cylindrical cell because the distance from the poles (blue spheres) to the site of cleavage is reduced, thereby shortening the time it takes the cleavage signal to reach the surface. (A,B: FROM CHARLES B. SHUSTER AND DAVID R. BURGESS, J. CELL BIOL. 146:987, 1999, FIG. 5. REPRODUCED WITH PERMISSION OF THE ROCKEFELLER UNIVERSITY PRESS.)

thus the plane of cytokinesis, is determined by a signal emanating from the spindle poles. The signal is thought to travel from the spindle poles to the cell cortex along the astral

microtubules. When the distance between the poles and the cortex is modified experimentally, the timing of cytokinesis can be dramatically altered (Figure 14.37). In contrast, researchers conducting studies on smaller mammalian cells have found evidence that the site of cleavage furrow formation is defined by a signal that originates in the central part of the mitotic spindle rather than its poles. Researchers have struggled to reconcile these opposing findings. The challenge is made greater by the fact that, unlike mitosis, cytokinesis has not been reconstituted in egg extracts, where it would be easiest to study. The simplest explanations are that (1) different cell types utilize different mechanisms or (2) both mechanisms operate in the same cell. In fact, recent studies provide evidence for this latter possibility.

Cytokinesis in Plant Cells: Formation of the Cell Plate

Plant cells, which are enclosed by a relatively inextensible cell wall, undergo cytokinesis by a very different mechanism. Unlike animal cells, which are constricted by a furrow that advances inward from the outer cell surface, plant cells must construct an extracellular wall inside a living cell. Wall formation starts in the center of the cell and grows outward to meet the existing lateral walls. The formation of a new cell wall begins with the construction of a simpler precursor, which is called the **cell plate**.

The plane in which the cell plate forms is perpendicular to the axis of the mitotic spindle but, unlike the case for animal cells, the plane is not determined by the position of the spindle nor is it determined late in mitosis. Rather, the orientation of both the mitotic spindle and cell plate are determined by a belt of cortical microtubules—the *preprophase band*—that forms in late G_2 (see Figure 9.21). Even though the preprophase band has disassembled by prometaphase, it leaves an invisible imprint that determines the future division site. The first sign of cell plate formation is seen in late anaphase with the appearance of the phragmoplast in the center of the dividing cell. The **phragmoplast** consists of clusters of interdigitating microtubules oriented perpendicular to the future plate (Figure 9.21), together with actin filaments, membranous vesicles, and electron-dense material. The microtubules of the phragmoplast, which arise from remnants of the mitotic spindle, serve as tracks for the movement of small Golgi-derived secretory vesicles into the region. The vesicles become aligned along a plane between the daughter nuclei (Figure 14.38*a*). Electron micrographs of rapidly frozen tobacco cells have revealed the steps by which the Golgi-derived vesicles become reorganized into the cell plate. To begin the process (step 1, Figure 14.38*b*), the vesicles send out finger-like tubules that contact and fuse with neighboring vesicles to

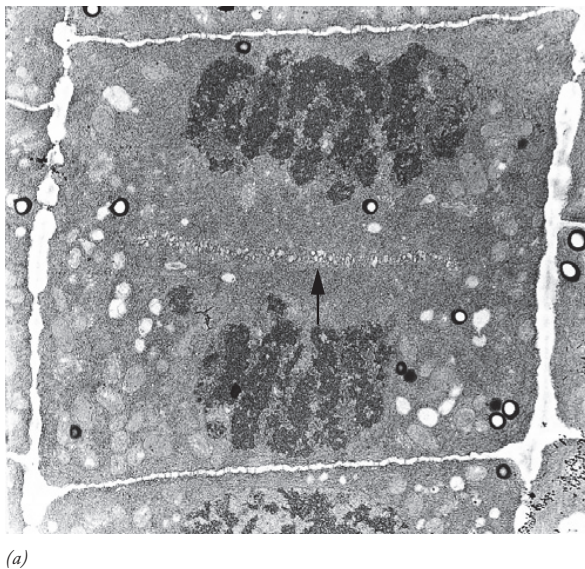
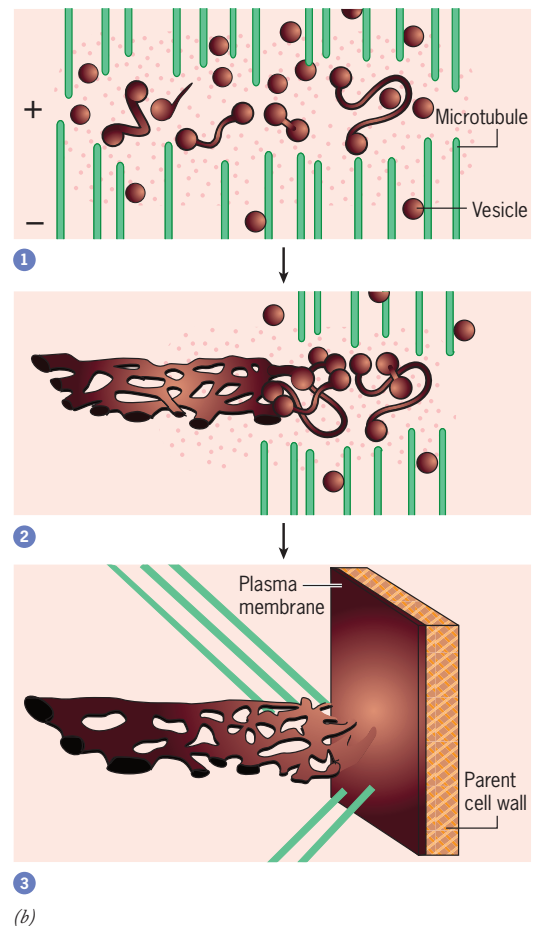


Figure 14.38 The formation of a cell plate between two daughter plant nuclei during cytokinesis. (a) A low-magnification electron micrograph showing the formation of the cell plate between the future daughter cells. Secretory vesicles derived from nearby Golgi complexes have become aligned along the equatorial plane (arrow) and are beginning to fuse with one another. The membrane of the vesicles forms the plasma membranes of the two daughter cells, and the contents of the vesicles will provide the material that forms the cell plate separating the cells. (b) Steps in the formation of the cell plate as described in the text. (A: DAVID PHILLIPS/PHOTO RESEARCHERS, INC.; B: A. L. SAMUELS, T. H. GIDDINGS, JR. & L. A. STAEHELIN, JOURNAL CELL BIOLOGY 130:1354, 1995. THE JOURNAL OF CELL BIOLOGY BY ROCKEFELLER INSTITUTE; AMERICAN SOCIETY FOR CELL BIOLOGY COPYRIGHT 1995 REPRODUCED WITH PERMISSION OF ROCKEFELLER UNIVERSITY PRESS IN THE FORMAT REPUBLISH IN A TEXTBOOK VIA COPYRIGHT CLEARANCE CENTER.)



form an interwoven tubular network in the center of the cell (step 2). Additional vesicles are then directed along microtubules to the lateral edges of the network. The newly arrived vesicles continue the process of tubule formation and fusion, which extends the network in an outward direction (step 2). Eventually, the leading edge of the growing network contacts the parent plasma membrane at the boundary of the cell (step 3). Ultimately, the tubular network loses its cytoplasmic gaps and matures into a continuous, flattened partition. The membranes of the tubular network become the plasma membranes of the two adjacent daughter cells, whereas the secretory products that had been carried within the vesicles contribute to the intervening cell plate. Once the cell plate is completed, cellulose and other materials are added to produce the mature cell wall.

REVIEW

1. How do the events of mitotic prophase prepare the chromatids for later separation at anaphase?
2. What are some of the activities of the kinetochore during mitosis?
3. Describe the events that occur in a cell during prometaphase and during anaphase.
4. Describe the similarities and differences in microtubule dynamics between metaphase and anaphase. How are the differences related to anaphase A and B movements?
5. What types of force-generating mechanisms might be responsible for chromosome movement during anaphase?
6. Contrast the events that occur during cytokinesis in typical plant and animal cells.

14.3 | Meiosis



The production of offspring by sexual reproduction includes the union of two cells, each with a haploid set of chromosomes.

As discussed in Chapter 10, the doubling of the chromosome number at fertilization is compensated by an equivalent reduction in chromosome number at a stage prior to formation of the gametes. This is accomplished by **meiosis**, a term coined in 1905 from the Greek word meaning “reduction.” Meiosis ensures production of a haploid phase in the life cycle, and fertilization ensures a diploid phase. Without meiosis, the chromosome number would double with each generation, and sexual reproduction would not be possible.

To compare the events of mitosis and meiosis, we need to examine the fate of the chromatids. Prior to both mitosis and meiosis, diploid G_2 cells contain pairs of homologous chromosomes, with each chromosome consisting of two chromatids. During mitosis, the chromatids of each chromosome are split apart and separate into two daughter nuclei in a *single* division. As a result, cells produced by mitosis contain pairs

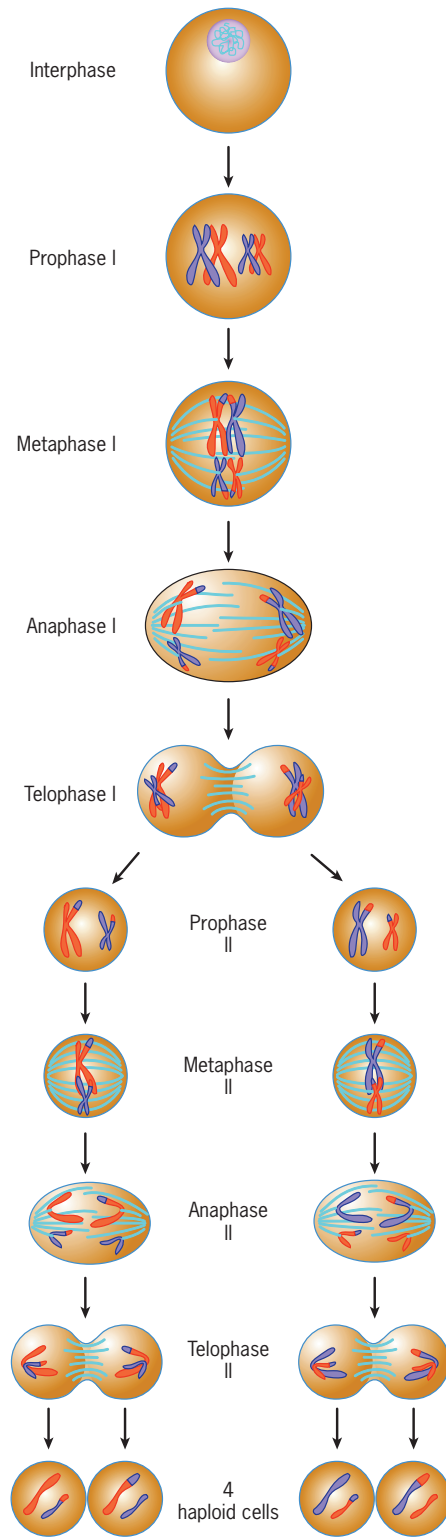


Figure 14.39 The stages of meiosis.

of homologous chromosomes and are genetically identical to their parents. During meiosis, in contrast, the four chromatids of a pair of replicated homologous chromosomes are distributed among four daughter nuclei. Meiosis accomplishes this

feat by incorporating two sequential divisions without an intervening round of DNA replication (Figure 14.39). In the first meiotic division, each chromosome (consisting of two chromatids) is separated from its homologue. As a result, each daughter cell contains only one member of each pair of homologous chromosomes. For this to occur, homologous chromosomes are paired during prophase of the first meiotic division (prophase I, Figure 14.39) by an elaborate process that has no counterpart in mitosis. As they are paired, homologous chromosomes engage in a process of genetic recombination that produces chromosomes with new combinations of

maternal and paternal alleles (see metaphase I, Figure 14.39). In the second meiotic division, the two chromatids of each chromosome are separated from one another (anaphase II, Figure 14.39).

A survey of various eukaryotes reveals marked differences with respect to the stage within the life cycle at which meiosis occurs and the duration of the haploid phase. The following three groups (Figure 14.40) can be identified on these bases:

1. **Gametic or terminal meiosis.** In this group, which includes all multicellular animals and many protists, the meiotic divisions are closely linked to the formation of the gametes (Figure 14.40, left). In male vertebrates (Figure 14.41a), for example, meiosis occurs just prior to the differentiation of the spermatozoa. *Spermatogonia* that are committed to undergo meiosis become *primary spermatocytes*, which then undergo the two divisions of meiosis to produce four relatively undifferentiated *spermatids*. Each spermatid undergoes a complex differentiation to become the highly specialized sperm cell (*spermatozoon*). In female vertebrates (Figure 14.41b), *oogonia* become *primary oocytes*, which then enter a greatly extended meiotic prophase. During this prophase, the primary oocyte grows and becomes filled with yolk and other materials. It is only after differentiation of the oocyte is complete (i.e., the oocyte has reached essentially the same state as when it is fertilized) that the meiotic divisions occur. Vertebrate eggs are typically fertilized at a stage before the completion of meiosis (usually at metaphase II). Meiosis is completed after fertilization, while the sperm resides in the egg cytoplasm.
2. **Zygotic or initial meiosis.** In this group, which includes only protists and fungi, the meiotic divisions occur just after fertilization (Figure 14.40, right) to produce haploid spores. The spores divide by mitosis to produce a haploid adult generation. Consequently, the diploid stage of the life cycle is restricted to a brief period after fertilization when the individual is still a zygote.
3. **Sporic or intermediate meiosis.** In this group, which includes plants and some algae, the meiotic divisions take place at a stage unrelated to either gamete formation or fertilization (Figure 14.40, center). If we begin the life cycle with the union of a male gamete (the pollen grain) and a female gamete (the egg), the diploid zygote undergoes mitosis and develops into a diploid **sporophyte**. At some stage in the development of the sporophyte, *sporogenesis* (which includes meiosis) occurs, producing spores that germinate directly into a haploid **gametophyte**. The gametophyte can be either an independent stage or, as in the case of seed plants, a tiny structure retained within the ovules. In either case, the gametes are produced from the haploid gametophyte by *mitosis*.

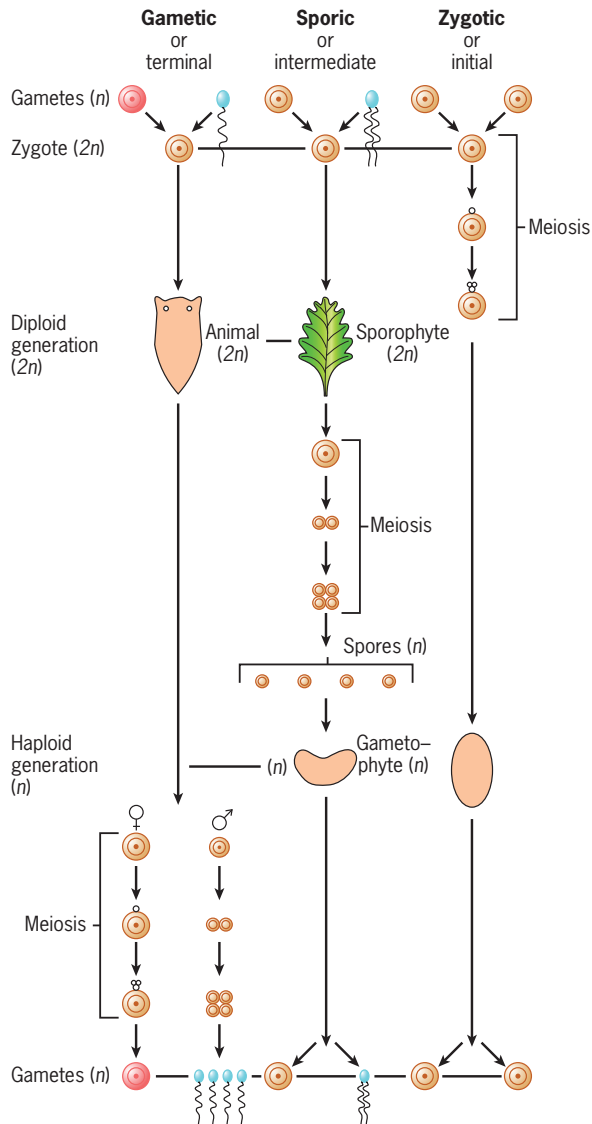


Figure 14.40 A comparison of three major groups of organisms based on the stage within the life cycle at which meiosis occurs and the duration of the haploid phase. (THE CELL IN DEVELOPMENT AND HEREDITY BY WILSON, EDMUND B. COPYRIGHT 1987. REPRODUCED WITH PERMISSION OF TAYLOR & FRANCIS GROUP LLC - BOOKS IN THE FORMAT TEXTBOOK VIA COPYRIGHT CLEARANCE CENTER.)

The Stages of Meiosis

As with mitosis, the prelude to meiosis includes DNA replication. The premeiotic S phase generally takes several times longer than a premitotic S phase. Prophase of the first meiotic

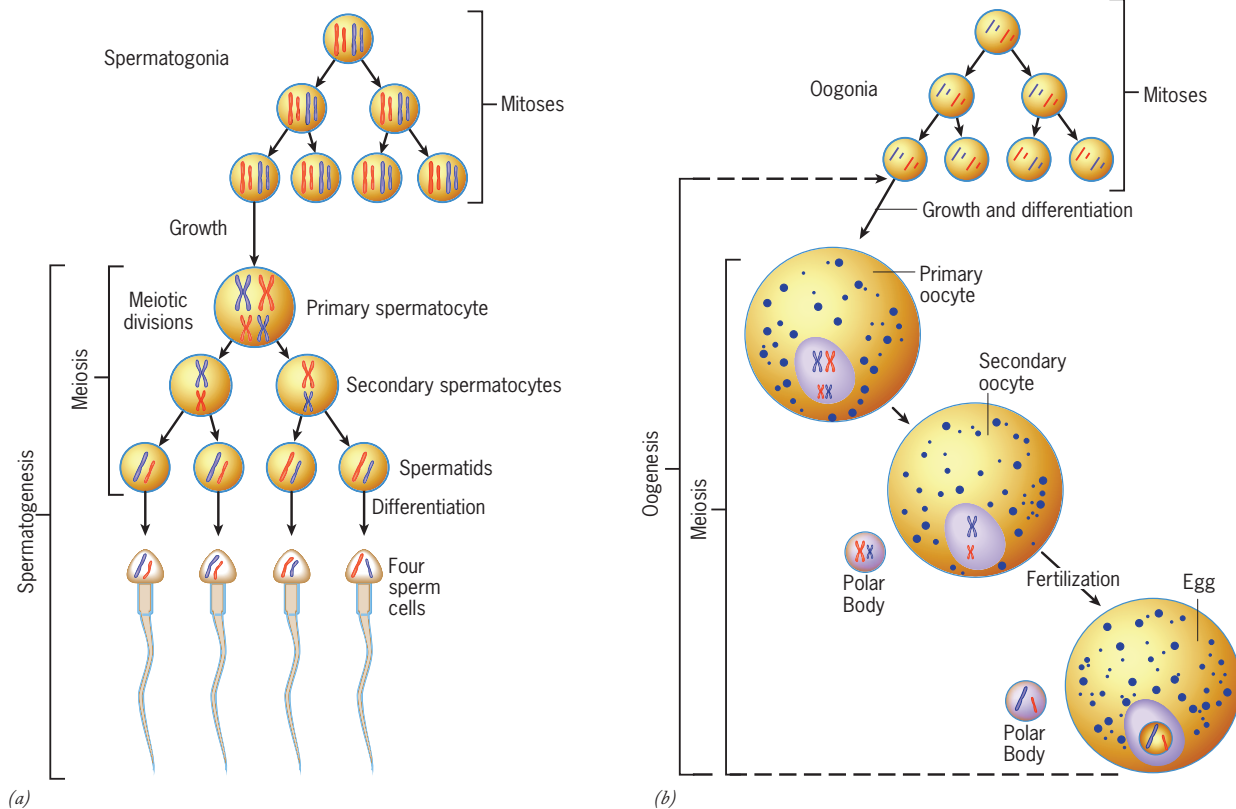


Figure 14.41 The stages of gametogenesis in vertebrates: a comparison between the formation of sperm and eggs. In both sexes, a relatively small population of primordial germ cells present in the embryo proliferates by mitosis to form a population of gonial cells (spermatogonia or oogonia) from which the gametes differentiate. In

the male (a), meiosis occurs before differentiation, whereas in the female (b), both meiotic divisions occur after differentiation. Each primary spermatocyte generally gives rise to four viable gametes, whereas each primary oocyte forms only one fertilizable egg and two or three polar bodies.

division (i.e., prophase I) is typically lengthened in extraordinary fashion when compared to mitotic prophase. In the human female, for example, oocytes initiate prophase I of meiosis prior to birth and then enter a period of prolonged arrest. Oocytes resume meiosis just prior to the time they are ovulated, which occurs every 28 days or so after an individual reaches puberty. Consequently, many human oocytes remain arrested in the same approximate stage of prophase for several decades. The first meiotic prophase is also very complex and is customarily divided into several stages that are similar in all sexually reproducing eukaryotes (Figure 14.42).

The first stage of prophase I is *leptotene*, during which the chromosomes become compacted and visible in the light microscope. Although the chromosomes have replicated at an earlier stage, there is no indication that each chromosome is actually composed of a pair of identical chromatids. In the electron microscope, however, the chromosomes are revealed to be composed of paired chromatids.

The second stage of prophase I, which is called *zygotene*, is marked by the visible association of homologues with one another. This process of chromosome pairing is called **synapsis** and is an intriguing event with important unanswered questions: On what basis do the homologues recognize one

another? How does the pair become so perfectly aligned? When does recognition between homologues first occur? Recent studies have shed considerable light on these questions. It had been assumed for years that interaction between homologous chromosomes first begins as chromosomes initiate synapsis. However, studies on yeast cells by Nancy Kleckner and her colleagues at Harvard University demonstrated that homologous regions of DNA from homologous chromosomes are already associated with one another during leptotene. Chromosome compaction and synapsis during zygotene simply make this arrangement visible under the microscope. As will be discussed below, the first step in genetic recombination is the deliberate introduction of double-stranded breaks in aligned DNA molecules. Studies in both yeast and mice suggest the DNA breaks occur in leptotene, well before the chromosomes are visibly paired.

These findings are supported by studies aimed at locating particular DNA sequences within the nuclei of premeiotic and meiotic cells. We saw on page 510 that individual chromosomes occupy discrete regions within nuclei rather than being randomly dispersed throughout the nuclear space. When yeast cells about to enter meiotic prophase are examined, each pair of homologous chromosomes is found to share a joint territory

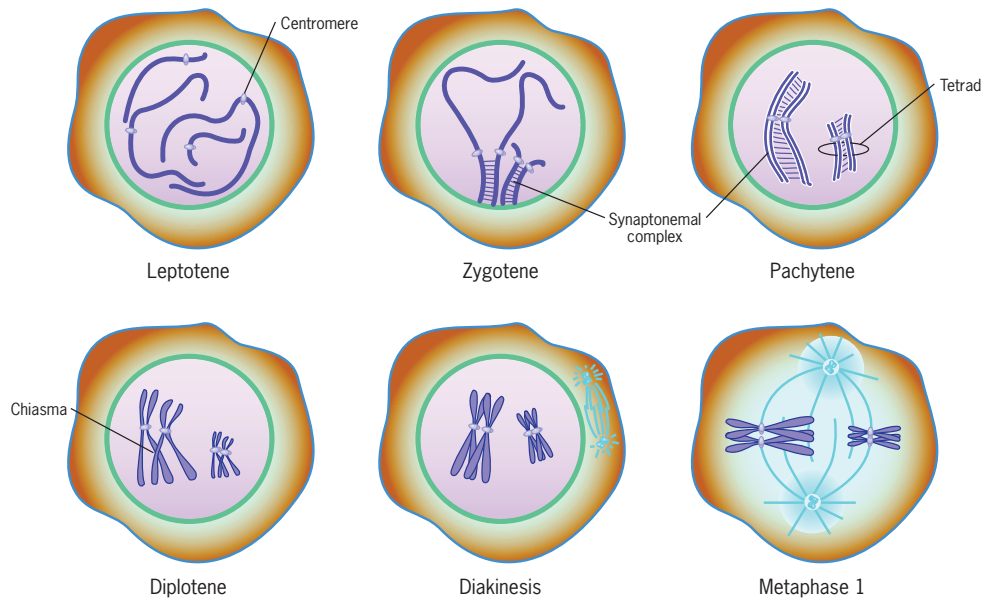


Figure 14.42 The stages of prophase I. The events at each stage are described in the text.

distinct from the territories shared by other pairs of homologues. This finding suggests that homologous chromosomes are paired to some extent before meiotic prophase begins. The



Figure 14.43 Association of the telomeres of meiotic chromosomes with the nuclear envelope. The chromosomes of a stage of meiotic prophase of the male grasshopper in which homologous chromosomes are physically associated as part of a bivalent. The bivalents are arranged in a well-defined “bouquet” with their terminal regions clustered near the inner surface of the nuclear envelope, at the base of the photo. (FROM B. JOHN, MEIOSIS, © 1990. CAMBRIDGE UNIVERSITY PRESS. REPRINTED WITH PERMISSION. COURTESY OF BERNARD JOHN.)

telomeres (terminal segments) of leptotene chromosomes are distributed throughout the nucleus. Then, near the end of leptotene, there is a dramatic reorganization of chromosomes in many species so that the telomeres become localized at the inner surface of the nuclear envelope on one side of the nucleus. Clustering of telomeres at one end of the nuclear envelope occurs in a wide variety of eukaryotes and causes the chromosomes to resemble the clustered stems of a bouquet of flowers (Figure 14.43). Mice carrying mutations that prevent the association of chromosomes with the nuclear envelope exhibit defects in synapsis, genetic recombination, and gamete formation. These experimental results suggest that the nuclear envelope plays an important role in the interaction between homologous chromosomes during meiosis.

Electron micrographs indicate that chromosome synapsis is accompanied by the formation of a complex structure called the synaptonemal complex. The **synaptonemal complex (SC)** is a ladder-like structure with transverse protein filaments connecting the two lateral elements (Figure 14.44). The chromatin of each homologue is organized into loops that extend from one of the lateral elements of the SC (Figure 14.44b). The lateral elements are composed primarily of cohesin (page 584), which presumably binds together the chromatin of the sister chromatids. For many years, the SC was thought to hold each pair of homologous chromosomes in the proper position to initiate genetic recombination between strands of homologous DNA. It is now evident that the SC is not required for genetic recombination. Not only does the SC form after genetic recombination has been initiated, but mutant yeast cells unable to assemble an SC can still engage in the exchange of genetic information between homologues. It is currently thought that the SC functions primarily as a scaffold to allow interacting chromatids to complete their crossover activities, as described below.

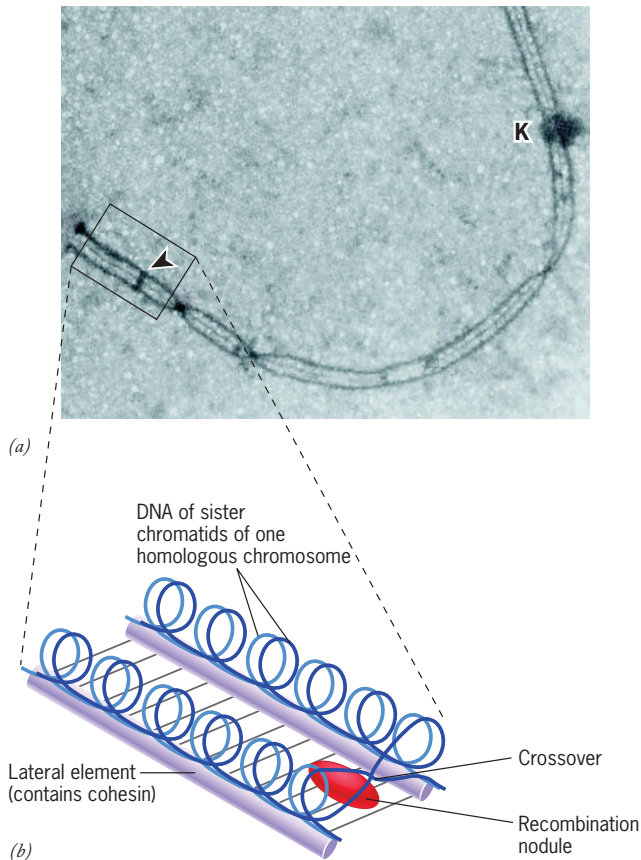


Figure 14.44 The synaptonemal complex. (a) Electron micrograph of a human pachytene bivalent showing a pair of homologous chromosomes held in a tightly ordered parallel array. K, kinetochore. (b) Schematic diagram of the synaptonemal complex and its associated chromosomal fibers. The dense granules (recombination nodules) seen in the center of the SC (indicated by the arrowhead in part a) contain the enzymatic machinery required to complete genetic recombination, which is thought to begin at a much earlier stage in prophase I. Closely paired loops of DNA from the two sister chromatids of each chromosome are depicted. The loops are likely maintained in a paired configuration by cohesin (not shown). Genetic recombination (crossing-over) is presumed to occur between the DNA loops from nonsister chromatids, as shown. (A: COURTESY OF ALBERTO J. SOLARI, CHROMOSOMA 81:330, 1980. WITH KIND PERMISSION FROM SPRINGER SCIENCE+ BUSINESS MEDIA.)

The complex formed by a pair of synapsed homologous chromosomes is called a **bivalent** or a **tetrad**. The former term reflects the fact that the complex contains two homologues, whereas the latter term calls attention to the presence of four chromatids. The end of synapsis marks the end of zygotene and the beginning of the next stage of prophase I, called *pachytene*, (Figure 14.44a), which is characterized by a fully formed synaptonemal complex. During pachytene, the homologues are held closely together along their length by the SC. The DNA of sister chromatids is extended into parallel loops (Figure 14.44b). Under the electron microscope, a number of

electron-dense bodies about 100 nm in diameter are seen within the center of the SC. These structures have been named *recombination nodules* because they correspond to the sites where crossing-over is taking place, as evidenced by the associated synthesis of DNA that occurs during intermediate steps of recombination (page 610). Recombination nodules contain the enzymatic machinery that facilitates genetic recombination, which is completed by the end of pachytene.

The beginning of *diplotene*, the next stage of meiotic prophase I (Figure 14.42), is recognized by the dissolution of the SC, which leaves the chromosomes attached to one another at specific points by X-shaped structures, termed **chiasmata** (singular **chiasma**) (Figure 14.45). Chiasmata are located at sites on the chromosomes where crossing-over between DNA molecules from the two chromosomes had previously occurred. Chiasmata are formed by covalent junctions between a chromatid from one homologue and a nonsister chromatid from the other homologue. These points of attach-

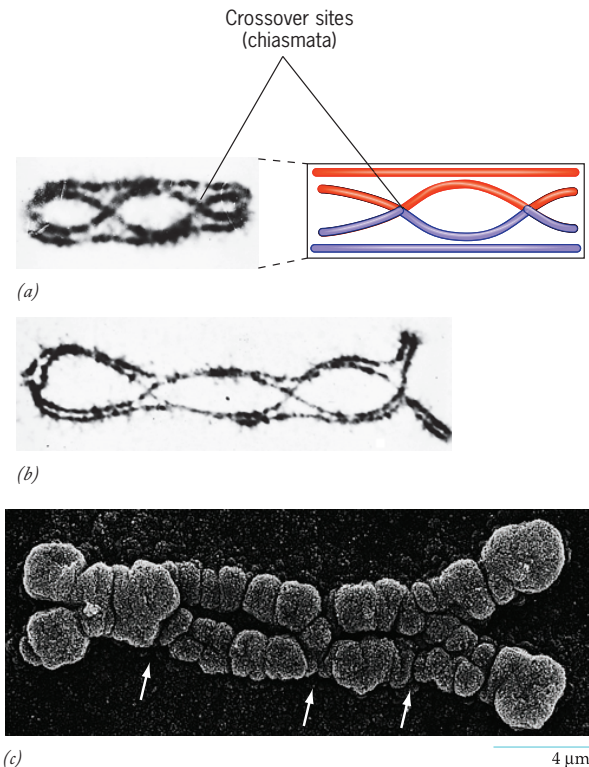


Figure 14.45 Visible evidence of crossing-over. (a,b) Diplotene bivalents from the grasshopper showing the chiasmata formed between chromatids of each homologous chromosome. The accompanying inset indicates the crossovers that have presumably occurred within the bivalent in a. The chromatids of each diplotene chromosome are closely apposed except at the chiasmata. (c) Scanning electron micrograph of a bivalent from the desert locust with three chiasmata (arrows). (A,B: FROM BERNARD JOHN, MEIOSIS, © 1990 CAMBRIDGE UNIVERSITY PRESS. REPRINTED WITH PERMISSION. C: FROM KLAUS WERNER WOLF, BIOESS. 16:108, 1994. REPRINTED WITH PERMISSION OF JOHN WILEY & SONS.)

ment provide a striking visual portrayal of the extent of genetic recombination. The chiasmata are made more visible by a tendency for the homologues to separate from one another at the diplotene stage.

In vertebrates, diplotene can be an extremely extended phase of oogenesis during which the bulk of oocyte growth occurs. Thus diplotene can be a period of intense metabolic activity. Transcription during diplotene in the oocyte provides the RNA utilized for protein synthesis during both oogenesis and early embryonic development following fertilization.

During the final stage of meiotic prophase I, called *diakinesis*, the meiotic spindle is assembled and the chromosomes are prepared for separation. In those species in which the chromosomes become highly dispersed during diplotene, the chromosomes become recompact during diakinesis. Diakinesis ends with the disappearance of the nucleolus, the breakdown of the nuclear envelope, and the movement of the tetrads to the metaphase plate. In vertebrate oocytes, these events are triggered by an increase in the level of the protein kinase activity of MPF (maturation-promoting factor). As discussed in the Experimental Pathways at the end of the chapter, MPF was first identified by its ability to initiate these events, which represent the *maturation* of the oocyte (page 611).

In most eukaryotic species, chiasmata can still be seen in homologous chromosomes aligned at the metaphase plate of meiosis I. In fact, chiasmata are required to hold the homologues together as a bivalent during this stage. In humans and other vertebrates, every pair of homologues typically contains at least one chiasma, and the longer chromosomes tend to have two or three of them. It is thought that some mechanism exists to ensure that even the smallest chromosomes form a chiasma. If a chiasma does not occur between a pair of homologous chromosomes, the chromosomes of that bivalent tend to separate from one another after dissolution of the SC. This premature separation of homologues often results in the formation of nuclei with an abnormal number of chromosomes. The consequences of such an event are discussed in the accompanying Human Perspective.

At metaphase I, the two homologous chromosomes of each bivalent are connected to the spindle fibers from opposite poles (Figure 14.46a). In contrast, sister chromatids are connected to microtubules from the same spindle pole, which is made possible by the side-by-side arrangement of their kinetochores as seen in the inset of Figure 14.46a. The orientation of the maternal and paternal chromosomes of each bivalent on the metaphase I plate is random; the maternal member of a particular bivalent has an equal likelihood of facing either pole. Consequently, when homologous chromosomes separate during anaphase I, each pole receives a random assortment of maternal and paternal chromosomes (see Figure 14.39). Thus, anaphase I is the cytological event that corresponds to Mendel's law of independent assortment (page 388). As a result of independent assortment, organisms are capable of generating a nearly unlimited variety of gametes.

Separation of homologous chromosomes at anaphase I requires the dissolution of the chiasmata that hold the bivalents together. The chiasmata are maintained by cohesion be-

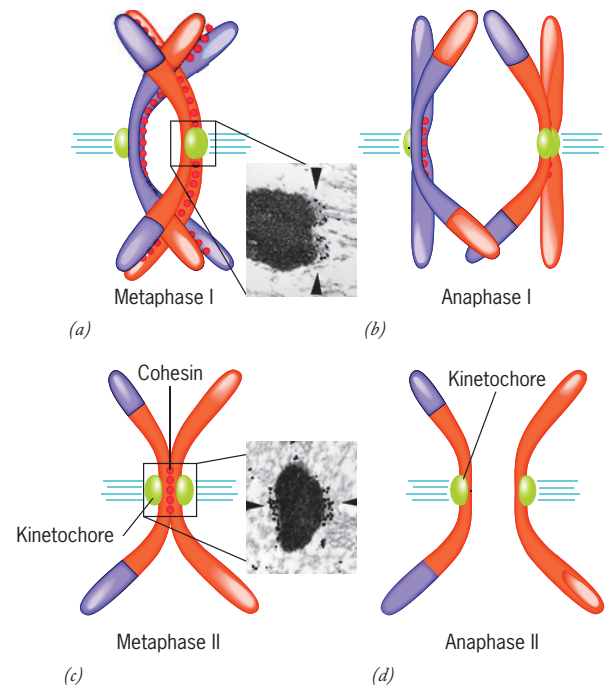


Figure 14.46 Separation of homologous chromosomes during meiosis I and separation of chromatids during meiosis II.

(a) Schematic diagram of a pair of homologous chromosomes at metaphase I. The chromatids are held together along both their arms and centromeres by cohesin. The pair of homologues are maintained as a bivalent by the chiasmata. Inset micrograph shows that the kinetochores (arrowheads) of sister chromatids are situated on one side of the chromosome, facing the same pole. The black dots are gold particles bound to the motor protein CENP-E of the kinetochores (see Figure 14.16c). (b) At anaphase I, the cohesin holding the arms of the chromatids is cleaved, allowing the homologues to separate from one another. Cohesin remains at the centromere, holding the chromatids together. (c) At metaphase II, the chromatids are held together at the centromere, with microtubules from opposite poles attached to the two kinetochores. Inset micrograph shows the kinetochores of the sister chromatids are now on opposite sides of the chromosome, facing opposite poles. (d) At anaphase II, the cohesin holding the chromatids together has been cleaved, allowing the chromosomes to move to opposite poles. (INSETS: FROM JIBAK LEE ET AL., MOL. REPROD. DEVELOP. 56:51, 2000. REPRINTED WITH PERMISSION OF JOHN WILEY & SONS.)

tween sister chromatids in regions that flank these sites of recombination (Figure 14.46a). The chiasmata disappear at the metaphase I–anaphase I transition, as the arms of the chromatids of each bivalent lose cohesion (Figure 14.46b). Loss of cohesion between the arms is accomplished by proteolytic cleavage of the cohesin molecules in those regions of the chromosome. In contrast, cohesion between the joined centromeres of sister chromatids remains strong, because the cohesin situated there is protected from proteolytic attack (Figure 14.46b). As a result, sister chromatids remain firmly attached to one another as they move together toward a spindle pole during anaphase I.

Telophase I of meiosis I produces less dramatic changes than telophase of mitosis. Although chromosomes often undergo some dispersion, they do not reach the extremely extended state of the interphase nucleus. The nuclear envelope may or may not reform during telophase I. The stage between the two meiotic divisions is called *interkinesis* and is generally short-lived. In animals, cells in this fleeting stage are referred to as *secondary spermatocytes* or *secondary oocytes*. These cells are characterized as being haploid because they contain only one member of each pair of homologous chromosomes. Even though they are haploid, they have twice as much DNA as a haploid gamete because each chromosome is still represented by a pair of attached chromatids. Secondary spermatocytes are said to have a 2C amount of DNA, half as much as a primary spermatocyte, which has a 4C DNA content, and twice as much as a sperm cell, which has a 1C DNA content.

Interkinesis is followed by prophase II, a much simpler prophase than its predecessor. If the nuclear envelope had reformed in telophase I, it is broken down again. The chromosomes become recompact and line up at the metaphase

plate. Unlike metaphase I, the kinetochores of sister chromatids of metaphase II face opposite poles and become attached to opposing sets of chromosomal spindle fibers (Figure 14.46c). The progression of meiosis in vertebrate oocytes stops at metaphase II. The arrest of meiosis at metaphase II is brought about by factors that inhibit APC^{Cdc20} activation, thereby preventing cyclin B degradation. As long as cyclin B levels remain high within the oocyte, Cdk activity is maintained, and the cells cannot progress to the next meiotic stage. Metaphase II arrest is released only when the oocyte (now called an egg) is fertilized. Fertilization leads to a rapid influx of Ca²⁺ ions, the activation of APC^{Cdc20} (page 592), and the destruction of cyclin B. The fertilized egg responds to these changes by completing the second meiotic division. Anaphase II begins with the synchronous splitting of the centromeres, which had held the sister chromatids together, allowing them to move toward opposite poles of the cell (Figure 14.46d). Meiosis II ends with telophase II, in which the chromosomes are once again enclosed by a nuclear envelope. The products of meiosis are haploid cells with a 1C amount of nuclear DNA.

THE HUMAN PERSPECTIVE

Meiotic Nondisjunction and Its Consequences



Meiosis is a complex process, and meiotic mistakes in humans appear to be surprisingly common. Homologous chromosomes may fail to separate from each other during meiosis I, or sister chromatids may fail to come apart during meiosis II. When either of these situations occurs, gametes are formed that contain an abnormal number of chromosomes—either an extra chromosome or a missing chromosome (Figure 1). If one of these gametes happens to fuse with a normal gamete, a zygote with an abnormal number of chromosomes forms, and serious consequences arise. In most cases, the zygote develops into an abnormal embryo that dies at some stage between conception and birth. In a few cases, however, the zygote develops into an infant whose cells have an abnormal chromosome number, a condition known as *aneuploidy*. The consequences of aneuploidy depend on which chromosome or chromosomes are affected.

The normal human chromosome complement is 46: 22 pairs of autosomes and one pair of sex chromosomes. An extra chromosome (producing a total of 47 chromosomes) creates a condition referred to as a *trisomy* (Figure 2). A person whose cells contain an extra chromosome 21, for example, has trisomy 21. A missing chromosome (producing a total of 45 chromosomes) produces a *monosomy*. We will begin by considering the effects of an abnormal number of autosomes.

Figure 1 Meiotic nondisjunction occurs when chromosomes fail to separate from each other during meiosis. If the failure to separate occurs during the first meiotic division, which is called *primary nondisjunction*, all of the haploid cells have an abnormal number of chromosomes. If nondisjunction occurs during the second meiotic division, which is called *secondary nondisjunction*, only two of the four haploid cells are affected. (More complex types of nondisjunction than shown here can also occur.)

The absence of one autosomal chromosome, regardless of which chromosome is affected, invariably proves to be lethal at some stage during embryonic or fetal development. Consequently, a zy-

