Learning Outcomes Review 10.2

Eukaryotic chromosomes are complex structures that can be compacted for cell division. During interphase, DNA is coiled around proteins into a structure called a nucleosome. The string of nucleosomes is further coiled into a solenoid (30-nm fiber). Diploid cells contain a maternal and paternal copy, or homologue, for each chromosome. After chromosome replication, each homologue consists of two sister chromatids. The chromatids are held together by proteins called cohesins.

- *Is chromosome number related to organismal complexity?*

10.3 Overview of the Eukaryotic Cell Cycle

Learning Outcome

1. *Describe the eukaryotic cell cycle.*

Compared with prokaryotes, the increased size and more complex organization of eukaryotic genomes required radical changes in the partitioning of replicated genomes into daughter cells. The **cell cycle** requires the duplication of the genome, its accurate segregation, and the division of cellular contents.

The cell cycle is divided into five phases

The cell cycle is divided into phases based on the key events of genome duplication and segregation. The cell cycle is usually diagrammed as in figure 10.8:

- **G_1 (gap phase 1)** is the primary growth phase of the cell. The term *gap phase* refers to its filling the gap between cytokinesis and DNA synthesis. For most cells, this is the longest phase.
- **S (synthesis)** is the phase in which the cell synthesizes a replica of the genome.
- **G_2 (gap phase 2)** is the second growth phase, and preparation for separation of the newly replicated genome. This phase fills the gap between DNA synthesis and the beginning of mitosis. During this phase microtubules begin to reorganize to form a spindle.

  G_1, S, and G_2 together constitute **interphase,** the portion of the cell cycle between cell divisions.
- **Mitosis** is the phase of the cell cycle in which the spindle apparatus assembles, binds to the chromosomes, and moves the sister chromatids apart. Mitosis is the essential step in the separation of the two daughter genomes. It is traditionally subdivided into five stages: prophase, prometaphase, metaphase, anaphase, and telophase.
- **Cytokinesis** is the phase of the cell cycle when the cytoplasm divides, creating two daughter cells. In animal cells, the microtubule spindle helps position a contracting ring of actin that constricts like a drawstring to pinch the cell in two. In cells with a cell wall, such as plant cells, a plate forms between the dividing cells.

Mitosis and cytokinesis together are usually referred to collectively as M phase, to distinguish the dividing phase from interphase.

The duration of the cell cycle varies depending on cell type

The time it takes to complete a cell cycle varies greatly. Cells in animal embryos can complete their cell cycle in under 20 min; the shortest known animal nuclear division cycles occur in fruit fly embryos (8 min). These cells simply divide their nuclei as quickly as they can replicate their DNA, without cell growth. Half of their cycle is taken up by S, half by M, and essentially none by G_1 or G_2.

Because mature cells require time to grow, most of their cycles are much longer than those of embryonic tissue. Typically, a dividing mammalian cell completes its cell cycle in about 24 hr, but some cells, such as certain cells in the human liver, have cell cycles lasting more than a year. During the cycle, growth occurs throughout the G_1 and G_2 phases, as well as during the S phase. The M phase takes only about an hour, a small fraction of the entire cycle.

Most of the variation in the length of the cell cycle between organisms or cell types occurs in the G_1 phase. Cells often pause in G_1 before DNA replication and enter a resting state called the **G_0 phase;** cells may remain in this phase for days to years before resuming cell division. At any given time, most of the cells in an

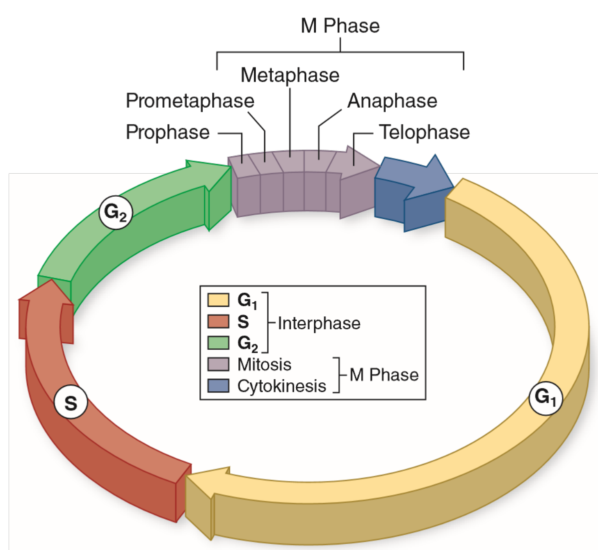


Figure 10.8 The cell cycle. The cell cycle is depicted as a circle. The first gap phase, G_1, involves growth and preparation for DNA synthesis. During S phase, a copy of the genome is synthesized. The second gap phase, G_2, prepares the cell for mitosis. During mitosis, replicated chromosomes are partitioned. Cytokinesis divides the cell into two cells with identical genomes.

animal's body are in G_0 phase. Some, such as muscle and nerve cells, remain there permanently; others, such as liver cells, can resume G_1 phase in response to factors released during injury.

Learning Outcome Review 10.3

Cell division in eukaryotes is a complex process that involves five phases: a first gap phase (G_1); a DNA synthesis phase (S); a second gap phase (G_2); mitosis (M), during which chromatids are separated; and cytokinesis in which a cell becomes two separate cells.

- ***When during the cycle is a cell irreversibly committed to dividing?***

10.4 Interphase: Preparation for Mitosis

Learning Outcomes

1. *Describe the events that take place during interphase.*
2. *Illustrate the connection between sister chromatids after S phase.*

The events that occur during interphase—the G_1, S, and G_2 phases—are very important for the successful completion of mitosis. During G_1, cells undergo the major portion of their growth. During the S phase, each chromosome replicates to produce two sister chromatids, which remain attached to each other at the centromere. In the G_2 phase, the chromosomes coil even more tightly.

The **centromere** is a point of constriction on the chromosome containing repeated DNA sequences that bind specific proteins. These proteins make up a disklike structure called the **kinetochore.** This disk functions as an attachment site for microtubules necessary to separate the chromosomes during cell division (figure 10.9). As seen in figure 10.6, each chromosome's centromere is located at a characteristic site along the length of the chromosome.

After the S phase, the sister chromatids appear to share a common centromere, but at the molecular level the DNA of the centromere has actually already replicated, so there are two complete DNA molecules. This means that two chromatids are held together by cohesin proteins at the centromere, and each chromatid has its own set of kinetochore proteins (figure 10.10). In multicellular animals, most of the cohesins that hold sister chromatids together after replication appear to be replaced by condensin as the chromosomes are condensed. This leaves the chromosomes still attached tightly at the centromere, but loosely attached elsewhere.

The cell grows throughout interphase. The G_1 and G_2 segments of interphase are periods of active growth, during which proteins are synthesized and cell organelles are produced. The cell's DNA replicates only during the S phase of the cell cycle.

After the chromosomes have replicated in S phase, they remain fully extended and uncoiled, although cohesin proteins are associated with them at this stage. In G_2 phase, they begin the

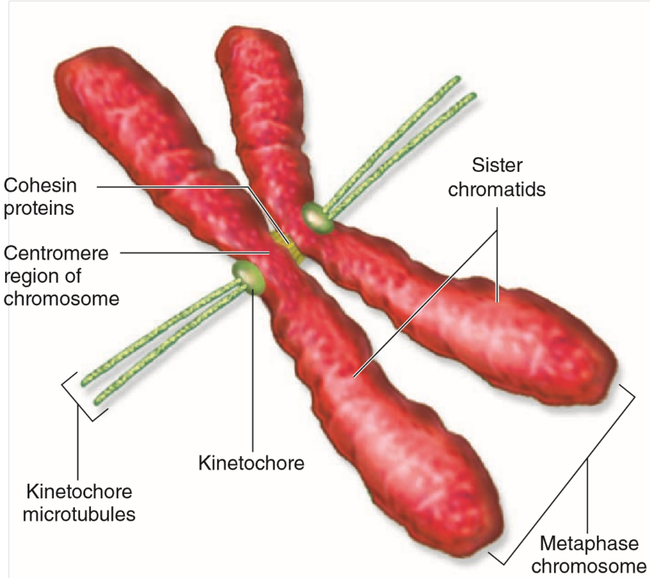


Figure 10.9 Kinetochores. Separation of sister chromatids during mitosis depends on microtubules attaching to proteins found in the kinetochore. These kinetochore proteins are assembled on the centromere of chromosomes. The centromeres of the two sister chromatids are held together by cohesin proteins.

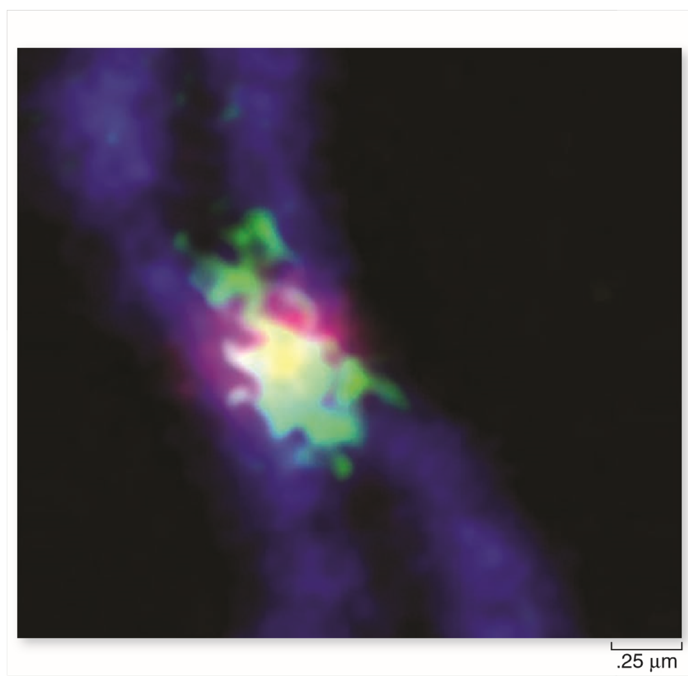


Figure 10.10 Proteins found at the centromere. In this image DNA, a human mitotic chromosome has been stained for condensin *(blue),* cohesin *(green)* and the centromere specific histone CENP-A *(red).* The cohesin proteins hold the centromeres of sister chromatids together.

process of condensation, coiling ever more tightly. Special *motor proteins* are involved in the rapid final condensation of the chromosomes that occurs early in mitosis. Also during G_2 phase, the cells begin to assemble the machinery they will later use to move the chromosomes to opposite poles of the cell. In animal cells, a pair of microtubule-organizing centers called *centrioles* replicate, producing one for each pole. All eukaryotic cells undertake an extensive synthesis of **tubulin,** the protein that forms microtubules.

Learning Outcomes Review 10.4

Interphase includes the G_1, S, and G_2 phases of the cell cycle. During interphase, the cell grows; replicates chromosomes, organelles, and centrioles; and synthesizes components needed for mitosis, including tubulin. Cohesin proteins hold chromatids together at the centromere of each chromosome.

- ***How would a mutation that deleted cohesin proteins affect cell division?***

10.5 M Phase: Chromosome Segregation and the Division of Cytoplasmic Contents

Learning Outcomes

1. *Describe the phases of mitosis.*
2. *Explain the importance of metaphase.*
3. *Compare cytokinesis in plants and animals.*

The process of mitosis is one of the most dramatic and beautiful biological processes that we can easily observe. In our attempts to understand this process, we have divided it into discrete phases but it should always be remembered that this is a dynamic, continuous process, not a set of discrete steps. This process is shown both schematically and in micrographs in figure 10.11.

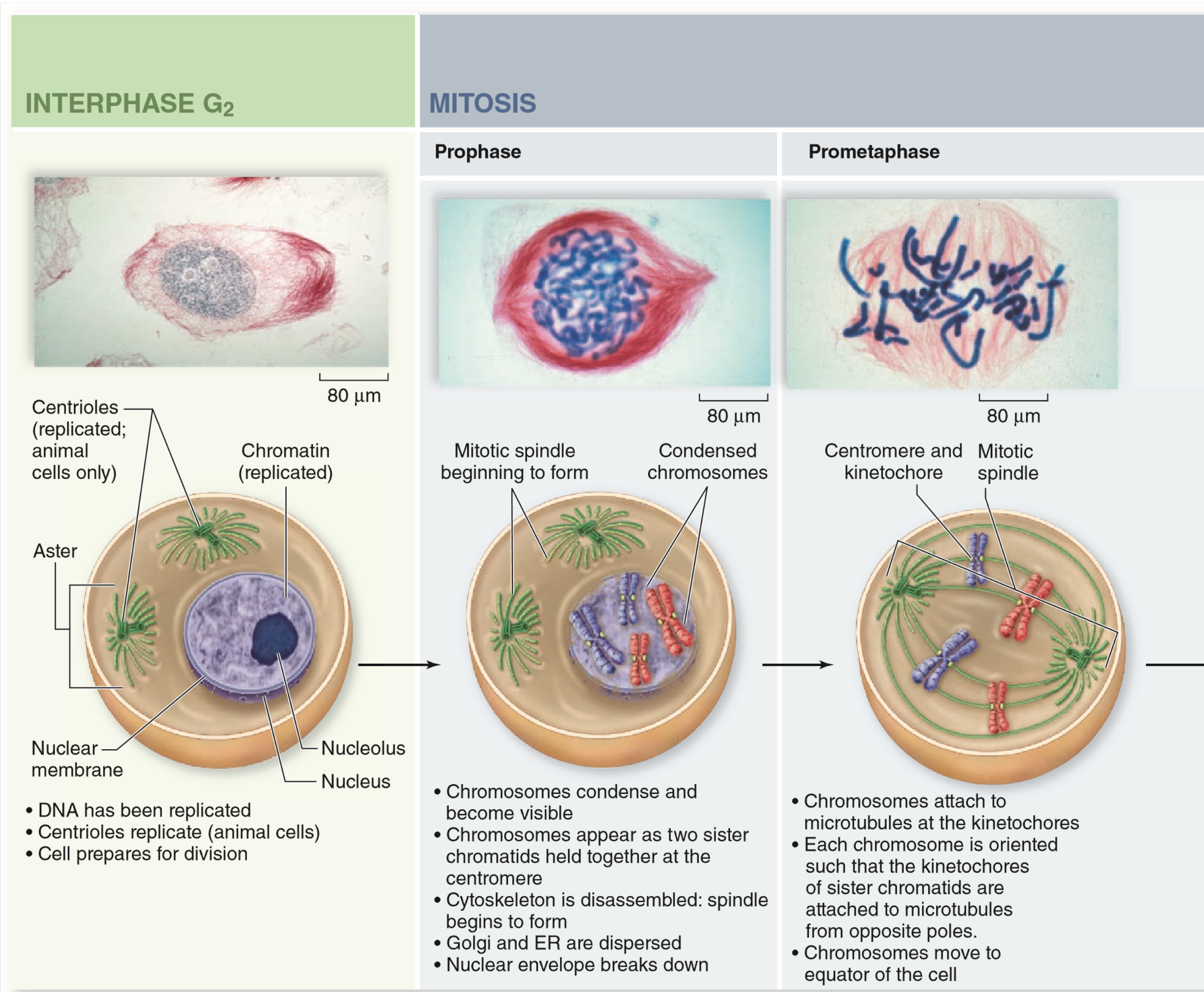


Figure 10.11 Mitosis and cytokinesis. Mitosis is conventionally divided into five stages—prophase, prometaphase, metaphase, anaphase, and telophase—which together act to separate duplicated chromosomes. This is followed by cytokinesis, which divides the cell into two separate cells. Photos depict mitosis and cytokinesis in a plant, the African blood lily *(Haemanthus katharinae),* with chromosomes stained blue and microtubules stained red. Drawings depict mitosis and cytokinesis in animal cells.

During prophase, the mitotic apparatus forms

When the chromosome condensation initiated in G_2 phase reaches the point at which individual condensed chromosomes first become visible with the light microscope, the first stage of mitosis, **prophase,** has begun. The condensation process continues throughout prophase; consequently, chromosomes that start prophase as minute threads appear quite bulky before its conclusion. Ribosomal RNA synthesis ceases when the portion of the chromosome bearing the rRNA genes is condensed.

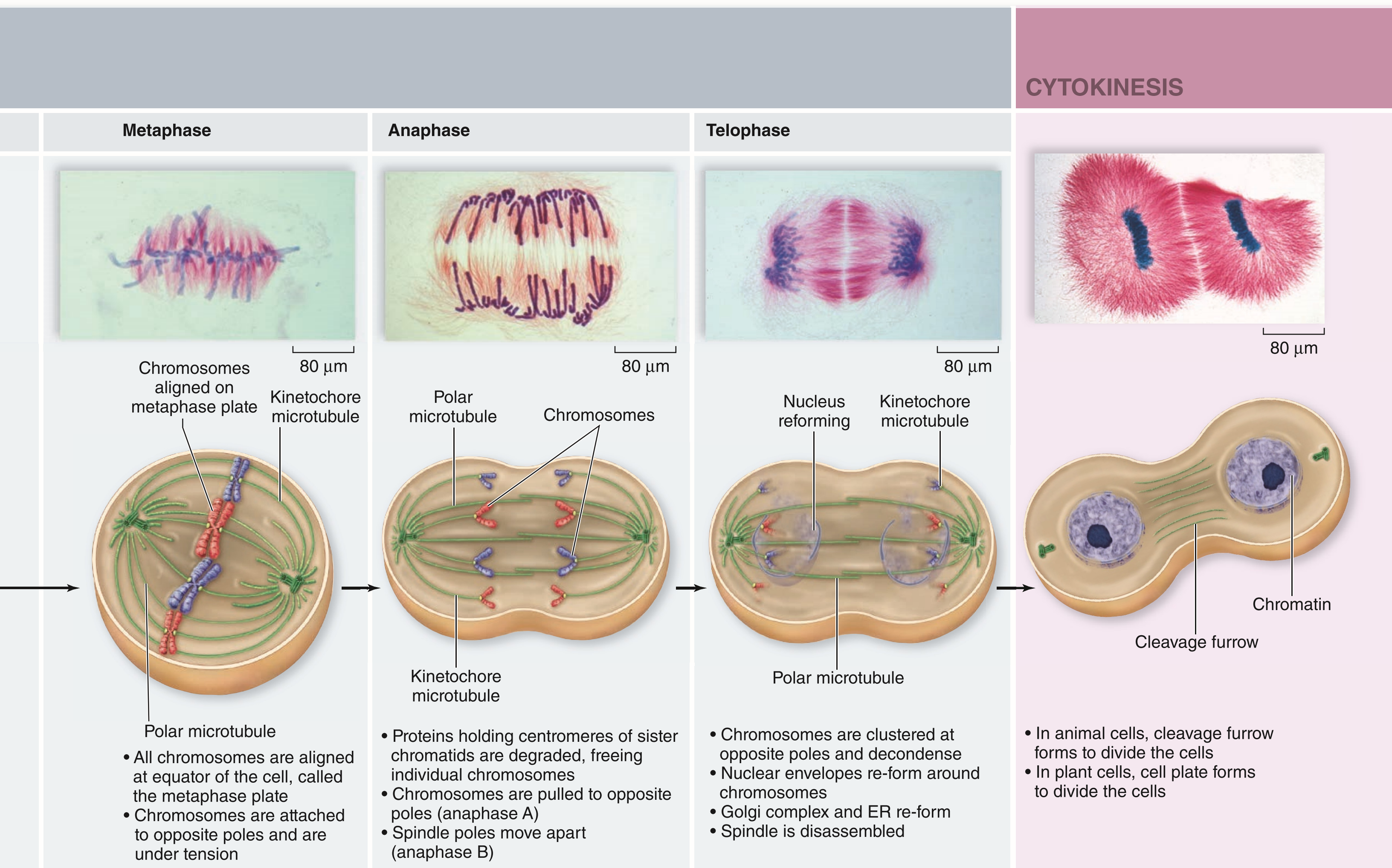
The spindle and centrioles

The assembly of the **spindle** apparatus that will later separate the sister chromatids occurs during prophase. The normal microtubule structure in the cell disassembled in the G_2 phase is replaced by the spindle. In animal cells, the two centriole pairs formed during G_2 phase begin to move apart early in prophase, forming between them an axis of microtubules referred to as spindle fibers. By the time the centrioles reach the opposite poles of the cell, they have established a bridge of microtubules, called the spindle apparatus, between them. In plant cells, a similar bridge of microtubular fibers forms between opposite poles of the cell, although centrioles are absent in plant cells.

In animal cell mitosis, the centrioles extend a radial array of microtubules toward the nearby plasma membrane when they reach the poles of the cell. This arrangement of microtubules is called an **aster.** Although the aster's function is not fully understood, it probably braces the centrioles against the membrane and stiffens the point of microtubular attachment during the retraction of the spindle. Plant cells, which have rigid cell walls, do not form asters.

Breakdown of the nuclear envelope

During the formation of the spindle apparatus, the nuclear envelope breaks down, and the endoplasmic reticulum reabsorbs its components. At this point, the microtubular spindle fibers extend completely across the cell, from one pole to the other. Their orientation determines the plane in which the cell will subsequently divide, through the center of the cell at right angles to the spindle apparatus.



During prometaphase, chromosomes attach to the spindle

The transition from prophase to **prometaphase** occurs following the disassembly of the nuclear envelope. During prometaphase the condensed chromosomes become attached to the spindle by their kinetochores. Each chromosome possesses two kinetochores, one attached to the centromere region of each sister chromatid (see figure 10.9).

Microtubule attachment

As prometaphase continues, a second group of microtubules grow from the poles of the cell toward the centromeres. These microtubules are captured by the kinetochores on each pair of sister chromatids. This results in the kinetochores of each sister chromatid being connected to opposite poles of the spindle.

This bipolar attachment is critical to the process of mitosis; any mistakes in microtubule positioning can be disastrous. For example, the attachment of the kinetochores of both sister chromatids to the same pole leads to a failure of sister chromatid separation, and they will be pulled to the same pole ending up in the same daughter cell, with the other daughter cell missing that chromosome.

Movement of chromosomes to the cell center

Each chromosome is attached to the spindle by microtubules running from opposite poles to the kinetochores of sister chromatids. The chromosomes are being pulled simultaneously toward each pole, leading to a jerky motion that eventually pulls all of the chromosomes to the equator of the cell. At this point, the chromosomes are arranged at the equator, with sister chromatids under tension and oriented toward opposite poles by their kinetochore microtubules.

The force that moves chromosomes has been of great interest since the process of mitosis was first observed. Two basic mechanisms have been proposed to explain this: (1) assembly and disassembly of microtubules provides the force to move chromosomes, and (2) motor proteins located at the kinetochore and poles of the cell pull on microtubules to provide force. Data have been obtained that support both mechanisms.

In support of the microtubule-shortening proposal, isolated chromosomes can be pulled by microtubule disassembly. The spindle is a very dynamic structure, with microtubules being added to at the kinetochore and shortened at the poles, even during metaphase. In support of the motor protein proposal, multiple motor proteins have been identified as kinetochore proteins, and inhibition of the motor protein dynein slows chromosome separation at anaphase. Like many phenomena that we analyze in living systems, the answer is not a simple either–or choice; both mechanisms are probably at work.

In metaphase, chromosomes align at the equator

The alignment of the chromosomes in the center of the cell signals the third stage of mitosis, **metaphase.** When viewed with a light microscope, the chromosomes appear to array themselves in a circle along the inner circumference of the cell, just as the equator girdles the Earth (figure 10.12). An imaginary plane perpendicular to the axis of the spindle that passes through this circle is called the *metaphase plate.* The metaphase plate is not an actual structure, but rather an indication of the future axis of cell division.

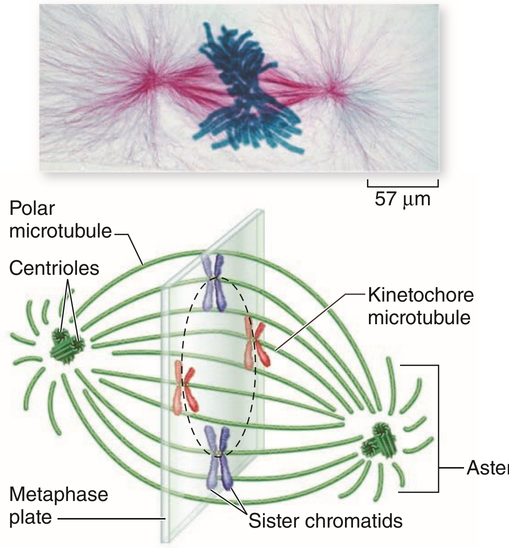


Figure 10.12 Metaphase. In metaphase, the chromosomes are arrayed at the midpoint of the cell. The imaginary plane through the equator of the cell is called the metaphase plate. As the spindle itself is a three-dimensional structure, the chromosomes are arrayed in a rough circle on the metaphase plate.

Positioned by the microtubules attached to the kinetochores of their centromeres, all of the chromosomes line up on the metaphase plate. At this point their centromeres are neatly arrayed in a circle, equidistant from the two poles of the cell, with microtubules extending back toward the opposite poles of the cell. The cell is prepared to properly separate sister chromatids, such that each daughter cell will receive a complete set of chromosomes. Thus metaphase is really a transitional phase in which all the preparations are checked before the action continues.

At anaphase, the chromatids separate

Of all the stages of mitosis, shown in figure 10.11, **anaphase** is the shortest and the most amazing to watch. It begins when the proteins holding sister chromatids together at the centromere are removed. Up to this point in mitosis, sister chromatids have been held together by cohesin proteins concentrated at the centromere, as described in section 10.2. The key event in anaphase, then, is the simultaneous removal of these proteins from all of the chromosomes. The control and details of this process are discussed in section 10.6 in the context of control of the entire cell cycle.

Freed from each other, the sister chromatids are pulled rapidly toward the poles to which their kinetochores are attached. In the process, two forms of movement take place simultaneously, each driven by microtubules. These movements are often called anaphase A and anaphase B to distinguish them.

First, during anaphase A, the *kinetochores are pulled toward the poles* as the microtubules that connect them to the poles shorten. This shortening process is not a contraction; the microtubules do not get any thicker. Instead, tubulin subunits are removed from the kinetochore ends of the microtubules. As more subunits are removed, the chromatid-bearing microtubules are progressively disassembled, and the chromatids are pulled ever closer to the poles of the cell.

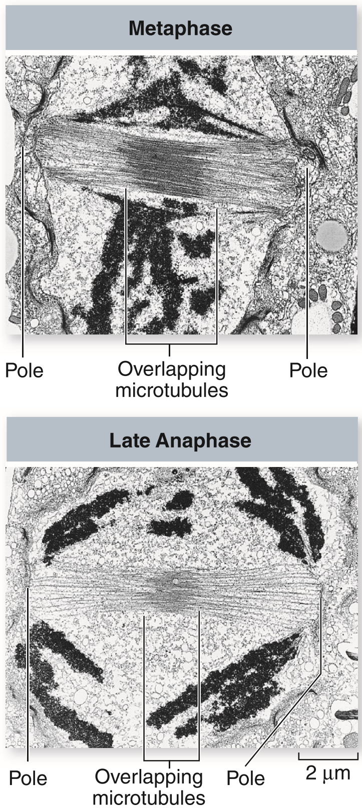


Figure 10.13 **Microtubules slide past each other as the chromosomes separate.** In these electron micrographs of dividing diatoms, the overlap of the microtubules lessens markedly during spindle elongation as the cell passes from metaphase to anaphase. During anaphase, B the poles move farther apart as the chromosomes move toward the poles.

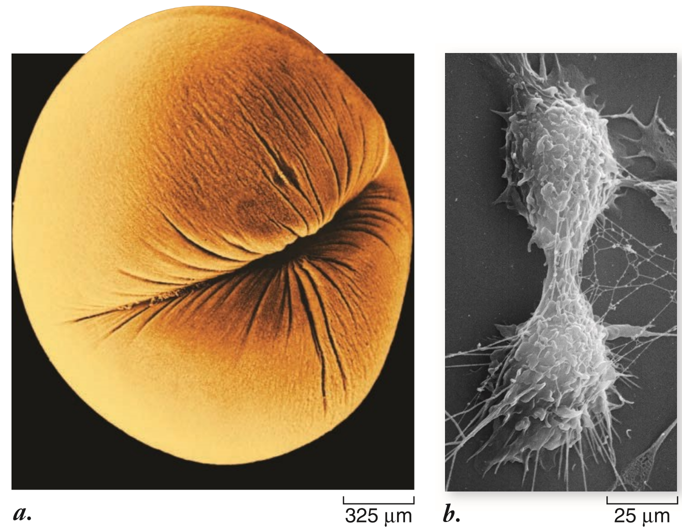


Figure 10.14 **Cytokinesis in animal cells.** ***a.*** A cleavage furrow forms around a dividing frog egg. ***b.*** The completion of cytokinesis in an animal cell. The two daughter cells are still joined by a thin band of cytoplasm occupied largely by microtubules.

Second, during anaphase B, the *poles move apart* as microtubular spindle fibers physically anchored to opposite poles slide past each other, away from the center of the cell (figure 10.13). Because another group of microtubules attach the chromosomes to the poles, the chromosomes move apart, too. If a flexible membrane surrounds the cell, it becomes visibly elongated.

When the sister chromatids separate in anaphase, the accurate partitioning of the replicated genome—the essential element of mitosis—is complete.

During telophase, the nucleus re-forms

In **telophase,** the spindle apparatus disassembles as the microtubules are broken down into tubulin monomers that can be used to construct the cytoskeletons of the daughter cells. A nuclear envelope forms around each set of sister chromatids, which can now be called chromosomes because they are no longer attached at the centromere. The chromosomes soon begin to uncoil into the more extended form that permits gene expression. One of the early group of genes expressed after mitosis is complete are the rRNA genes, resulting in the reappearance of the nucleolus.

Telophase can be viewed as a reversal of the process of prophase, bringing the cell back to the state of interphase. Mitosis is complete at the end of telophase. The eukaryotic cell has partitioned its replicated genome into two new nuclei positioned at opposite ends of the cell. Other cytoplasmic organelles, including mitochondria and chloroplasts (if present), were reassorted to areas that will separate and become the daughter cells.

Cell division is still not complete at the end of mitosis, however, because the division of the cell body proper has not yet begun. The phase of the cell cycle when the cell actually divides is called **cytokinesis.** It generally involves the cleavage of the cell into roughly equal halves.

In animal cells, a belt of actin pinches off the daughter cells

In animal cells and the cells of all other eukaryotes that lack cell walls, cytokinesis is achieved by means of a constricting belt of actin filaments. As these filaments slide past one another, the diameter of the belt decreases, pinching the cell and creating a **cleavage furrow** around the cell's circumference (figure 10.14*a*).

As constriction proceeds, the furrow deepens until it eventually slices all the way into the center of the cell. At this point, the cell is divided in two (figure 10.14*b*).

In plant cells, a cell plate divides the daughter cells

Plant cell walls are far too rigid to be squeezed in two by actin filaments. Instead, these cells assemble membrane components in their interior, at right angles to the spindle apparatus. This expanding membrane partition, called a **cell plate,** continues to grow outward until it reaches the interior surface of the plasma membrane and fuses with it, effectively dividing the cell in two (figure 10.15). Cellulose is then laid down on the new membranes, creating two new cell walls. The space between the daughter cells becomes impregnated with pectins and is called a *middle lamella.*

In fungi and some protists, daughter nuclei are separated during cytokinesis

In most fungi and some groups of protists, the nuclear membrane does not dissolve, and as a result, all the events of mitosis occur entirely *within* the nucleus. Only after mitosis is complete in these