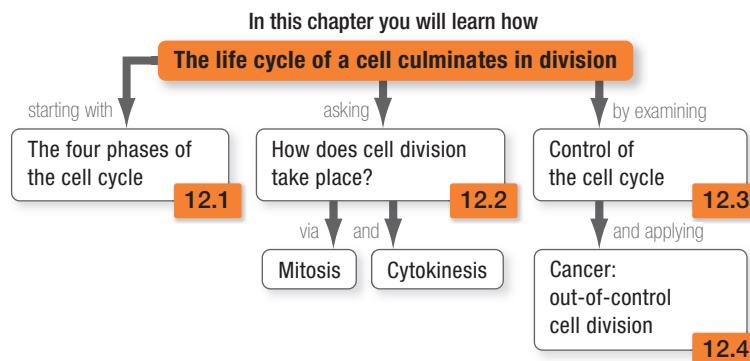




Pr. G. Giménez-Martín/Photo Researchers, Inc./Science Source

12 The Cell Cycle

This cell, from a hyacinth plant, is undergoing a type of nuclear division called mitosis. Understanding how mitosis occurs is a major focus of this chapter.



The cell theory maintains that all organisms are made of cells and that all cells arise from preexisting cells (Chapter 1). Although the cell theory was widely accepted among biologists by the 1860s, most thought that new cells arose within preexisting cells by a process that resembled the growth of mineral crystals. But Rudolf Virchow proposed that new cells are formed by the splitting of preexisting cells—that is, by **cell division**.

In the late 1800s, microscopic observations of newly developing organisms, or **embryos**, confirmed Virchow's hypothesis. Plants and animals start life as single-celled embryos and grow through a series of cell divisions.

Early studies revealed two fundamentally different ways that nuclei divide before cell division: meiosis and mitosis. In animals, **meiosis** leads to the production of sperm and eggs, which are the male and female reproductive cells termed **gametes**. Meiosis is equally important in other eukaryotes, but the cells produced are not gametes. In plants, for example, the products of meiosis are **spores**. **Mitosis** leads to the production of the other cell types, referred to as **somatic** (literally, “body-belonging”) **cells**. (You can see how meiosis and mitosis are related to each other and to the transmission of genetic information in the Big Picture on pages 408–409.)

BIG PICTURE

This chapter is part of the Big Picture. See how on pages 408–409.

Mitosis and meiosis are usually accompanied by **cytokinesis**—the division of the cytoplasm into two distinct cells. When cytokinesis is complete, a so-called parent cell has given rise to two daughter cells.

Mitotic and meiotic cell divisions are responsible for one of the five fundamental attributes of life: reproduction (see Chapter 1). But even though mitosis and meiosis share many characteristics, they are fundamentally different. During mitotic division, the genetic material is copied and then divided equally between two cells. This is referred to as cellular *replication*, since the daughter cells are genetically identical to the parent cell. In contrast, meiosis results in daughter cells that are genetically different from each other and that have half the amount of hereditary material as the parent cell.

This chapter focuses on mitotic cell division; meiotic cell division is the subject of another chapter (Chapter 13). Let's begin with a look at the key events in a cell's life cycle, continue with an in-depth analysis of mitosis and the regulation of the cell cycle, and end by examining how uncontrolled cell division can lead to cancer.

12.1 How Do Cells Reproduce?

For life on Earth to exist, cells must replicate. The basic steps in cellular replication are (1) copying the DNA (deoxyribonucleic acid), (2) separating the copies, and (3) dividing the cytoplasm to create two complete cells. This chapter focuses on a process that has been studied for well over a century: how eukaryotic cells replicate. Like much work in biology, the research on eukaryotic cell replication began with simple observations of the process.

What Is a Chromosome?

As studies of cell division in eukaryotes began, biologists found that certain chemical dyes made thread-like structures visible within nuclei. In 1879, Walther Flemming used a dye made from a coal tar to observe these structures and watch them change in the dividing cells of salamander embryos. The threads first appeared in pairs just before cell division and then split to produce single, unpaired threads in the daughter cells. Flemming introduced the term "mitosis," from the Greek *mitos* ("thread"), to describe this process.

Others studied the roundworm *Ascaris* and noted that the number of threads in a cell was the same before and after mitotic division. All of these cells had the same number of threads.

In 1888, Wilhelm Waldeyer coined the term **chromosome** ("coloured body") to refer to these thread-like structures (visible in the chapter-opening photo). Research carried out since then has shown that a chromosome consists of a single long DNA double helix that is wrapped around proteins, called **histones**, in a highly organized manner (see Chapter 19). DNA encodes the cell's hereditary information, or genetic material. A **gene** is a region of DNA in a chromosome that codes for a particular protein or ribonucleic acid (RNA).

Before mitosis, each chromosome is replicated. As mitosis starts, the chromosomes condense into compact structures that can be moved around the cell efficiently. Then one copy of each chromosome is distributed to each of two daughter cells.

Figure 12.1 illustrates an unreplicated chromosome, the same chromosome after it has been replicated, and the replicated chromosome that has condensed at the start of mitosis. Each of the double-stranded DNA copies in a replicated chromosome is called a **chromatid**. The two chromatids are held together by proteins at a large DNA region called the **centromere** ("centre part"). Centromeres are often, but not always, found in the

Unreplicated chromosome

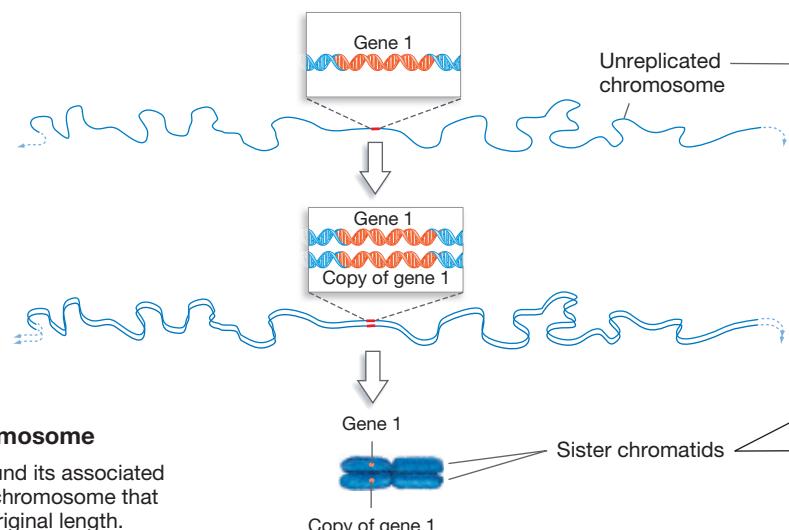
Consists of a single, long DNA double helix wrapped around proteins (which are too small to distinguish at this scale).

Replicated chromosome

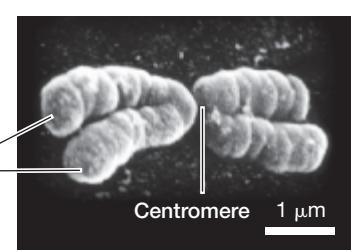
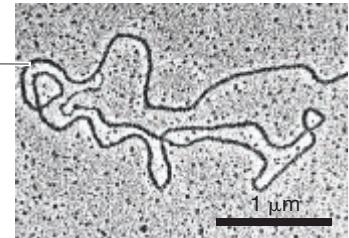
Consists of two copies of the same DNA double helix.

Condensed replicated chromosome

Consists of DNA condensed around its associated proteins, resulting in a compact chromosome that is 10 000 times shorter than its original length.



Biophoto Associates/Photo Researchers, Inc./Science Source



Dr. Gopal Murti/Photo Researchers, Inc./Science Source

Figure 12.1 Changes in Chromosome Morphology. After chromosomes replicate, the two identical copies of the double-stranded DNA are attached to each other along their entire length. Early in mitosis, replicated chromosomes condense and sister chromatids remain attached at a region called the centromere.

middle of chromosomes. Even though a replicated chromosome consists of two chromatids, it is still considered a single chromosome. During mitosis the “sister” chromatids are separated, at which time they become “daughter” chromosomes.

✓ If you understand how a chromosome can be made of one or two pieces of DNA, you should be able to draw a model to represent a cell with two different chromosomes before and after the chromosomes are replicated. Use a circle to represent the cell and one of the models above to represent the chromosomes. Label the chromatids.

Cells Alternate between M Phase and Interphase

The division of eukaryotic cells is like a well-choreographed stage performance. The most visually stimulating part of the show occurs when cells are in the process of separating their chromosomes, called **M (mitotic or meiotic) phase**. Stained chromosomes can be observed with a light microscope when they condense into compact structures during M phase.

The rest of the time, the cell is in **interphase** (“between phase”). No dramatic changes in the nucleus are visible by light microscopy during interphase. The chromosomes uncoil into the extremely long, thin structures shown in Figure 12.1 and no longer appear as individual threads. However, this does not mean that the cell is idle. Interphase is an active time: The cell is either growing and preparing to divide or fulfilling its specialized function in a multicellular individual. Cells actually spend most of their time in interphase.

The Discovery of S Phase

Once M phase and interphase were identified by microscopy, researchers could start assigning roles to these distinct phases. They could see that the separation of chromosomes and cytokinesis take place during M phase, but when are the chromosomes replicated?

To answer this question, researchers needed to distinguish cells that were making copies of their DNA from those that were not. They were able to do this by adding radioactive phosphorus, in the form of phosphates, to cells. Those cells that were synthesizing DNA would incorporate the radioactive isotope into nucleotides. (See Chapter 4 to review where phosphates are in DNA.) There were three steps in this procedure:

1. Label DNA as chromosomes were being replicated.
2. Wash away any radioactive phosphorus that hadn't been incorporated and remove RNA, which would also incorporate phosphorus.
3. Visualize the radioactive, newly synthesized DNA by exposing the treated cells to X-ray film. Emissions from radioactive phosphorus create black dots in the film.

The scientists who found success with this technique were Alma Howard and Stephen Pelc. Howard was born in Montreal and earned her Ph.D. at McGill before moving to England to collaborate with Pelc. In 1951, they performed this procedure and found black dots—indicating active DNA synthesis—in some interphase cells, but not in M-phase cells. This result showed that

DNA replication occurs during a period in interphase. Several years later, this result was verified using radioactive thymidine, which is incorporated into DNA but not RNA.

Thus, biologists had identified a new stage in the life of a cell. They called it **S (or synthesis) phase**. S phase is part of interphase. The process of copying the genetic material is separated, in time, from the partitioning of replicated chromosomes during M phase.

Howard and Pelc coined the term **cell cycle** to describe the orderly sequence of events that leads a eukaryotic cell through the duplication of its chromosomes to the time it divides.

The Discovery of the Gap Phases

In addition to discovering S phase, Howard and Pelc made another key observation—not all interphase cells were radioactive. This meant that there was at least one “gap” in interphase when DNA was not being replicated.

Howard and Pelc, along with researchers in other labs, followed up on these early results by asking where S phase was positioned in interphase. There were three possible scenarios:

1. The cell cycle has a single gap between the end of M phase and the start of S phase.
2. The cell cycle has a single gap between the end of S phase and the start of M phase.
3. Two gaps exist, one before and one after S phase.

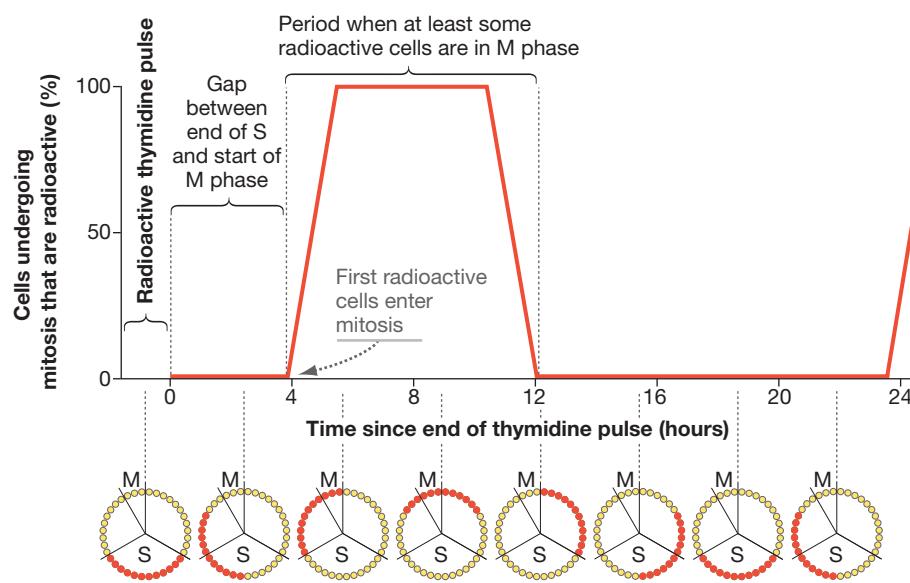
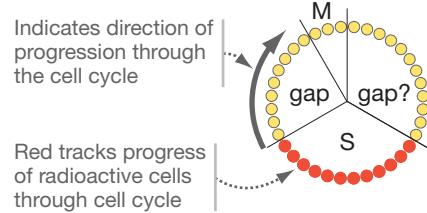
To address which of these scenarios, if any, was correct, many experiments were done on cells in culture. Cultured cells are powerful experimental tools because they can be manipulated much more easily than cells in an intact organism (see **BioSkills 9**). In most of these experiments, researchers used cultures that were *asynchronous*, meaning that the cells were randomly distributed in various stages of the cell cycle.

To understand the value of asynchronous cultures, imagine the cell cycle as a clock. Every complete rotation of the second hand around the clock would represent one cell division, and each tick would represent a different point in the cycle. At any given time, an asynchronous culture would have at least one cell at each of the ticks on the clock. As time passed, these cells would move around this cell-cycle clock at the same rate and in the same direction.

In one experiment, researchers added radioactively labelled thymidine to the cells in a human cell culture. A short time later, they stopped the process by flooding the solution surrounding the cultured cells with nonradioactive thymidine, which washed away any radioactive thymidine that had not already been incorporated into DNA. This pulse–chase approach (introduced in Chapter 7) labelled only those cells that were in S phase during the radioactive pulse. Imagine these radioactive cells moving together through the cell cycle like the second hand moving around a clock.

Once the pulse ended, the researchers took samples of cells from the culture at different times during the chase. In each sample, they recorded how many radioactive cells were undergoing mitosis, meaning how many cells that were in S phase during the pulse had entered M phase. **Figure 12.2** summarizes the results of this experiment.

Figure 12.2 A Pulse–Chase Experiment Reveals a Gap Phase. Cells labelled with radioactive thymidine during the pulse were tracked during the chase. The period between the end of the pulse and the appearance of the first radioactive mitotic cells represents a gap between the end of S phase and start of M phase.



One striking result emerged early on: None of the radioactive cells started mitosis immediately. Because the cultures were asynchronous, at least some of the cells must have been at the very end of their S phase when they were exposed to the pulse. If S phase were immediately followed by M phase, then some of these radioactive cells would have entered M phase just as the chase began. Instead, it took several hours before any of the radioactive cells began mitosis.

The time between the end of the pulse and the appearance of the first radioactive mitotic nuclei corresponds to a gap between the end of S phase and the beginning of M phase. This gap is a period when chromosome replication is complete but mitosis has not yet begun. The graph in Figure 12.2 shows how cells labelled with radioactive thymidine can be tracked as they progress through M phase.

✓ **If you understand how the pulse–chase approach was used in Figure 12.2, you should be able to predict how the graph would appear if the y-axis represented the percentage of all cells that were radioactive, not just the radioactive cells undergoing mitosis.**

This result narrowed the possible scenarios for the organization of the cell cycle: There could be either one gap between the end of S phase and the start of M phase, or two gaps flanking S phase. Which scenario represents the eukaryotic cell cycle? Once researchers determined the lengths of the S and M phases, they found that the combined time, including the gap between them, was shorter than the length of the cell cycle. This discrepancy indicated that there must be an additional gap between the end of M phase and the start of S phase.

The cell cycle was thus finally mapped out. The gap between the end of M phase and the start of S phase is called **G₁ phase**. The second gap, between the end of S phase and the start of M phase, is called **G₂ phase**.

The Cell Cycle

Figure 12.3 pulls these results together into a comprehensive view of the cell cycle. The cell cycle involves four phases: M phase

and an interphase consisting of the G₁, S, and G₂ phases. In the cycle diagrammed here, G₁ phase is about twice as long as G₂ phase, but their actual durations vary depending on the cell type and growth conditions.

Why do the gap phases exist? In multicellular organisms, cells perform their functional roles mostly during G₁ phase. G₁ is also the period when the cell “decides” to begin replication and transitions to S phase (as will be explained in Section 12.3). Before mitosis can take place, a cell uses G₂ phase to prepare for M phase. The time spent in both G₁ and G₂ allows the cell to grow and replicate organelles so it will be able to divide into two cells that can function normally.

Now let’s turn to M phase. Once the genetic material has been copied in S phase, how is it divided between daughter cells?

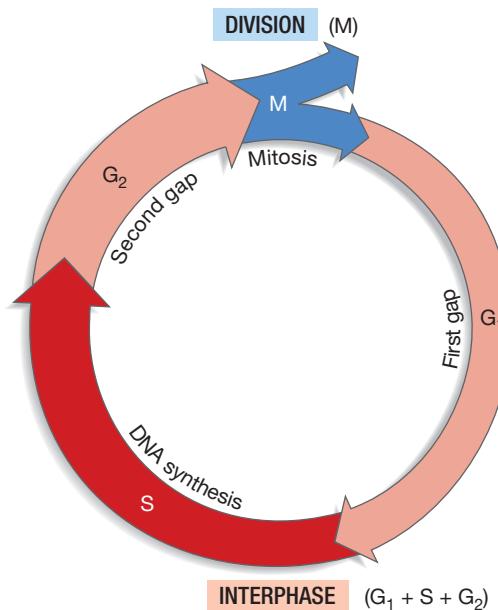


Figure 12.3 The Cell Cycle Has Four Phases. The duration of the G₁ and G₂ phases varies dramatically among cells and organisms.

12.2 What Happens during M Phase?

The purpose of mitosis is to ensure that each daughter cell inherits a nucleus containing one copy of each chromosome. Curiously, the total number of chromosomes varies considerably from species to species. Fruit flies have 8, people have 46, and dogs have 78. Later in this chapter, we will follow a hypothetical animal cell with a total of four chromosomes.

Chromosomes in most cells are wrapped around globular histone proteins. This DNA-protein complex is called **chromatin**. As we will discuss, DNA is always associated with histone proteins but is often covered with additional proteins.

If eukaryotic cells had much smaller chromosomes and they were found in the cytosol instead of within an organelle, mitosis would be a simple task. At the appropriate signal, each replicated chromosome would be split into its two chromatids. Each piece of DNA, now a true chromosome, could be moved to one end of the cell or the other. Once there was one copy of each chromosome at both ends of the cell, cytokinesis could occur.

Proteins Needed for Mitosis

Cohesins How could the cell make these events occur? First, there must be some way of holding the sister chromatids together until the signal is given. This is achieved with a large number of protein rings called **cohesins**. **Figure 12.4a** shows how these rings, made of three smaller proteins joined end to end, can encircle two pieces of DNA. At the signal, the rings would be cleaved and the sister chromatids would then be independent chromosomes.

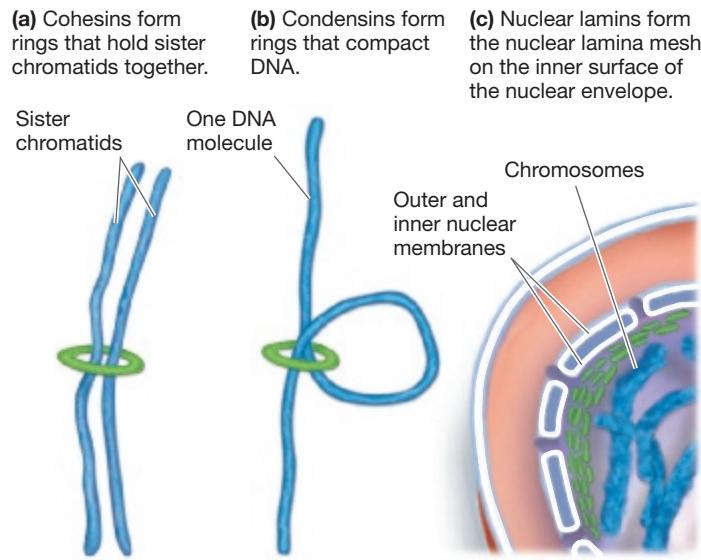


Figure 12.4 Cohesins, Condensins, and Nuclear Lamins

Each Play an Important Role during Mitosis. Because cohesin and condensin proteins are tiny in comparison with entire chromosomes, it takes thousands of cohesins to hold the sister chromatids together and thousands of condensins to compact each chromosome in preparation for cell division.

Microtubules The second problem to overcome is moving the chromosomes to the poles. The solution is to use **microtubules**. Recall from Chapter 7 that microtubules are usually used to transport vesicles and move flagella; here, they will be used to move pieces of DNA.

Kinetochore Proteins The sites where microtubules connect to the chromosomes are known as the **kinetochores** ("movement places"). Kinetochore proteins form an interface between the DNA and the microtubules. In most eukaryotes, the kinetochore proteins and the cohesin proteins mentioned earlier are found at the same site on the chromosome, the centromere.

✓ If you understand the two functions of the centromere region you should be able to show where the microtubule proteins will connect to the chromosome shown in the bottom right corner of **Figure 12.1**.

Nuclear Lamins One difference between our hypothetical cell and an actual cell is that the chromosomes are normally within the nucleus, while the microtubules are found in the cytosol. How can the microtubules connect to the chromosomes?

The solution is for the cell to temporarily dismantle the nuclear envelope. In Chapter 7, you also learned that what gives the nucleus its shape is a mesh of intermediate filaments called the **nuclear lamina**. It forms an interface between the chromosomes and the inside of the nuclear envelope, as seen in **Figure 12.4c**. If these large protein fibres were to be temporarily separated into their component **nuclear lamins**, the nuclear envelope would pull away from the chromosomes and withdraw into the endoplasmic reticulum. Problem solved.

Condensins The other difference between our hypothetical cell and an actual cell is the length of the chromosomes. An average human chromosome contains a piece of DNA 75 mm long! Even when wrapped around histone proteins, the chromosome is 2 mm long. While this is acceptable in the interphase nucleus, it would be impossible during cell division to move 46 chromosomes to each pole if each was several times longer than the cell was wide.

The solution to this third problem is that, at the beginning of cell division, the chromosomes must become more condensed. This is done with proteins called **condensins**. As seen in **Figure 12.4b**, they, like cohesins, are ring-shaped; like cohesins, they are also made of three subunits. They also encircle DNA, but instead of holding two different pieces together, they stabilize loops in the same piece of DNA. Condensin proteins allow our chromosomes to be about 5 μm long during mitosis (Figure 12.1).

There are other proteins involved in mitosis, some of which will be introduced later in this chapter. However, at its simplest level, mitosis is a story of DNA working together with five proteins: cohesins, microtubules, kinetochore proteins, nuclear lamins, and condensins.

Events in Mitosis

Although mitosis is a continuous process, biologists identify five subphases within M phase on the basis of distinctive events

that occur. Some students use the mnemonic device IPPMAT to remember that interphase is followed by the mitotic subphases of prophase, prometaphase, metaphase, anaphase, and telophase.

While mitosis is a complex process, keep in mind its function. At the start of mitosis the cell has a single nucleus and at the end it has two. Mitosis is the replication of a eukaryotic cell's most complex organelle—its nucleus. This is an essential step that must occur before it can divide into two independent cells.

Interphase To successfully complete mitosis a cell needs to have completed three tasks: (1) DNA replication, (2) cell growth, and (3) microtubule-organizing centre (MTOC) replication (**Figure 12.5**, step 1). We've discussed the first two events already in this chapter but what of MTOC replication? Recall from Chapter 7 that microtubules are often connected at their bases to MTOCs. Animal and fungal cells have single MTOCs called **centrosomes**. Centrosomes are large protein complexes that contain small bundles of microtubules known as centrioles. Plant cells have hundreds of smaller MTOCs.

An animal cell that is committed to cell division replicates its centrosome in the cytosol at the same time as it replicates its DNA within its nucleus. The result is the cell shown in **Figure 12.5**, step 1—the chromosomes are replicated and there are two centrosomes. The centrosomes will play important roles at each step of mitosis and each daughter cell will end up inheriting one.

Plant cells don't have centrosomes but they still need to organize their microtubules during mitosis. Scientists studying the model plant *Arabidopsis* (see **BioSkills 9**) recently found out how this occurs. This plant, and presumably others like it, uses a protein called NEDD1. Just before mitosis begins these proteins accumulate on the surface of the nuclear envelope. They form two patches on opposite sides of the nucleus. Microtubules then radiate outwards from these complexes. These structures remain in place for the rest of mitosis, even after the nuclear envelope is temporarily dismantled. NEDD1 protein complexes are not as large as centrosomes but are similar in composition and perform many of the same tasks during mitosis. Other organisms, including animals, use NEDD1 proteins in various MTOCs.

Prophase Mitosis begins with the events of **prophase** ("before phase"; **Figure 12.5**, step 2), when chromosomes condense into compact structures. Chromosomes first become visible in the light microscope during prophase.

In the cytoplasm, prophase is marked by the formation of the spindle apparatus. The spindle apparatus is a structure that produces mechanical forces that (1) pull chromosomes to the poles of the cell during mitosis and (2) push the poles of the cell away from each other.

The **spindle apparatus** consists of distinct populations of microtubules anchored at their base to a centrosome (in an animal cell) or NEDD1 complex (in a plant cell). Depending upon the cell type there will be two or three types:

1. **Polar microtubules** extend outwards and overlap with other polar microtubules attached to the opposite MTOC.
2. **Kinetochore microtubules** connect to chromosomes.

3. **Astral microtubules** are found only in animal cells and connect the centrosome to proteins on the inner surface of the plasma membrane.

During prophase in animal cells, the centrosomes move away from each other. In plants and other eukaryotes, the MTOCs are already at opposite sides of the cell.

Prometaphase As the chromosomes become completely condensed, the nuclear envelope begins to disappear. Microtubules extend into the middle of the cell. Some contact chromosomes and become kinetochore microtubules, while others contact microtubules coming from the opposite side of the cell and become polar microtubules. These events occur during **prometaphase** ("before middle phase"; **Figure 12.5**, step 3).

Note how the kinetochore regions on each chromosome appear as a constriction. It can't be seen in this figure but there are two kinetochores on each chromosome, one on each chromatid. Microtubules from each pole can only attach to one chromatid or the other.

Early in mitosis, kinesin and dynein motors are recruited to the kinetochore, where they can "walk" the chromosome up and down microtubules. These motors are thought to be very important in the initial attachment of the kinetochore to the plus end of the microtubule. If these ideas are correct, then the process is similar to the way these motors walk along microtubules during vesicle transport (see Chapter 7).

In all eukaryotes, after the kinetochores have attached to microtubules, chromosomes begin to move to the middle of the cell during prometaphase.

Metaphase Once the kinetochore microtubules have moved all the chromosomes to the middle of the spindle (**Figure 12.5**, step 4), the mitotic cells enter **metaphase** ("middle phase"). At this point, the chromosomes are lined up along an imaginary plane between the two spindle poles called the **metaphase plate**.

The formation of the spindle apparatus is now complete. The polar microtubules that extend from each spindle pole overlap in the middle of the cell, thereby forming a pole-to-pole connection. Each chromosome is held by kinetochore microtubules reaching out from opposite poles and exerting the same amount of tension, or pull. In animal cells, the centrosomes are held in place by the astral microtubules that interact with proteins on the cell membrane.

The alignment of these chromosomes results from the growth and shrinkage of the attached kinetochore microtubules. When chromosomes reach the metaphase plate, the shrinkage of these microtubules at the MTOCs is balanced by slow growth of microtubules at the kinetochores. Since the sister chromatids of each chromosome are connected to opposite poles, a tug of war occurs during metaphase that pulls them in opposite directions.

Anaphase At the start of **anaphase** ("against phase"), the cohesins that are holding sister chromatids together at the centromeres split (**Figure 12.5**, step 5). Because the chromatids are under tension, each replicated chromosome is pulled apart to create two independent daughter chromosomes. By definition, this separation of chromatids instantly doubles the number of chromosomes in the cell.

PROCESS: MITOSIS

Sister chromatids separate; one chromosome copy goes to each daughter nucleus.

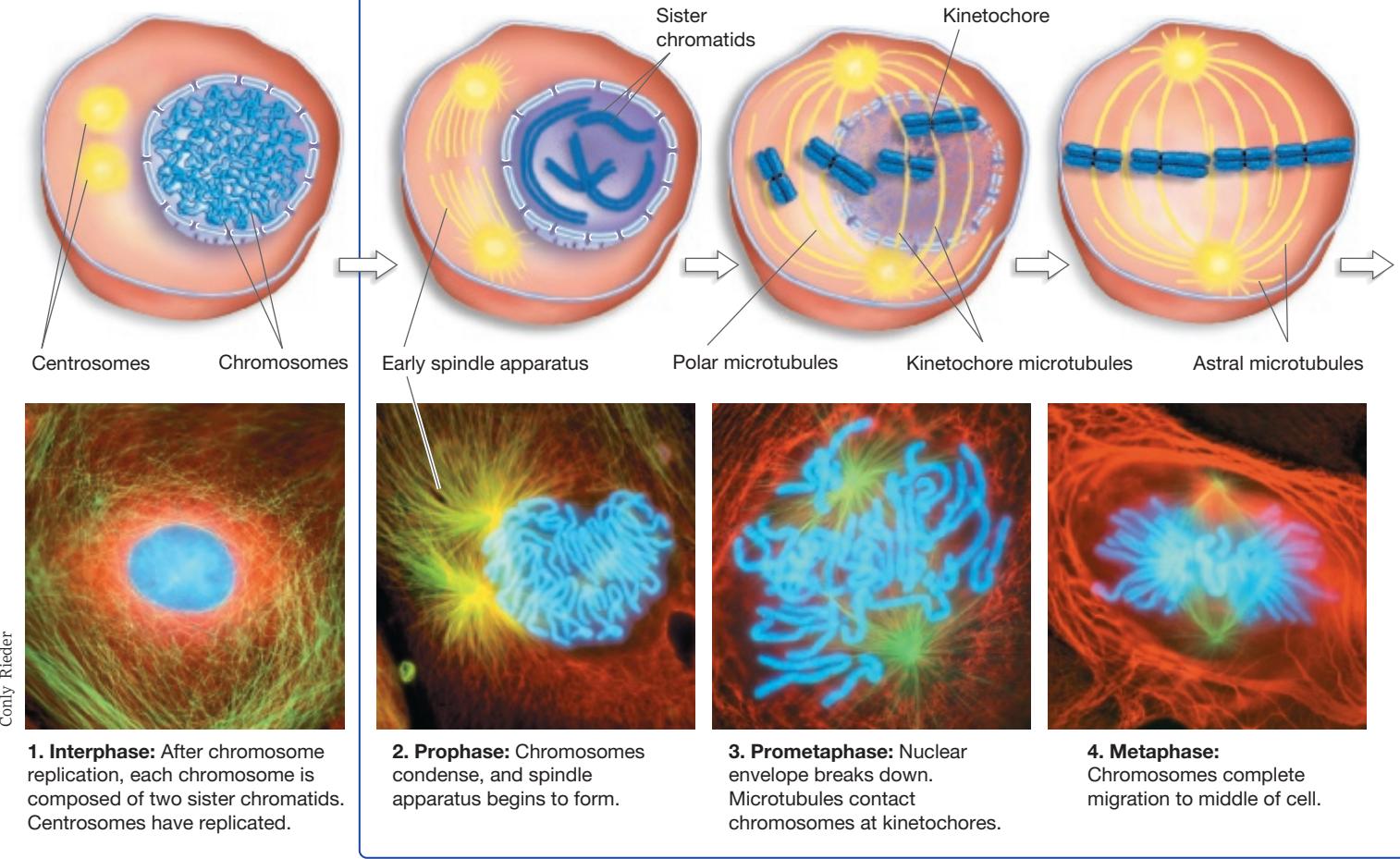


Figure 12.5 Mitosis and Cytokinesis. In the micrographs of newt lung cells under the drawings, chromosomes are stained blue, microtubules are yellow/green, and intermediate filaments are red.

◀ CAUTION If the cell shown in the micrographs has 60 picograms of DNA (6×10^{-11} g) and 22 chromosomes in its G₁ phase, how much DNA and how many chromosomes are in (1) the prophase cell, (2) the anaphase cell, and (3) each daughter cell?

Two types of movement occur during anaphase. First, the daughter chromosomes move to opposite poles via the attachment of kinetochore proteins to the shrinking kinetochore microtubules. Second, the two poles of the spindle are pushed and pulled farther apart. The motor proteins in overlapping polar microtubules push the poles away from each other. Different motors on the membrane walk along on the astral microtubules to pull the poles to opposite sides of the cell.

During anaphase, then, replicated chromosomes split into two identical sets of daughter chromosomes. Their separation to opposite poles is a critical step in mitosis because it ensures that each daughter cell receives the same complement of chromosomes.

When anaphase is complete, two complete collections of chromosomes are fully separated, each identical with those of the parent cell before chromosome replication.

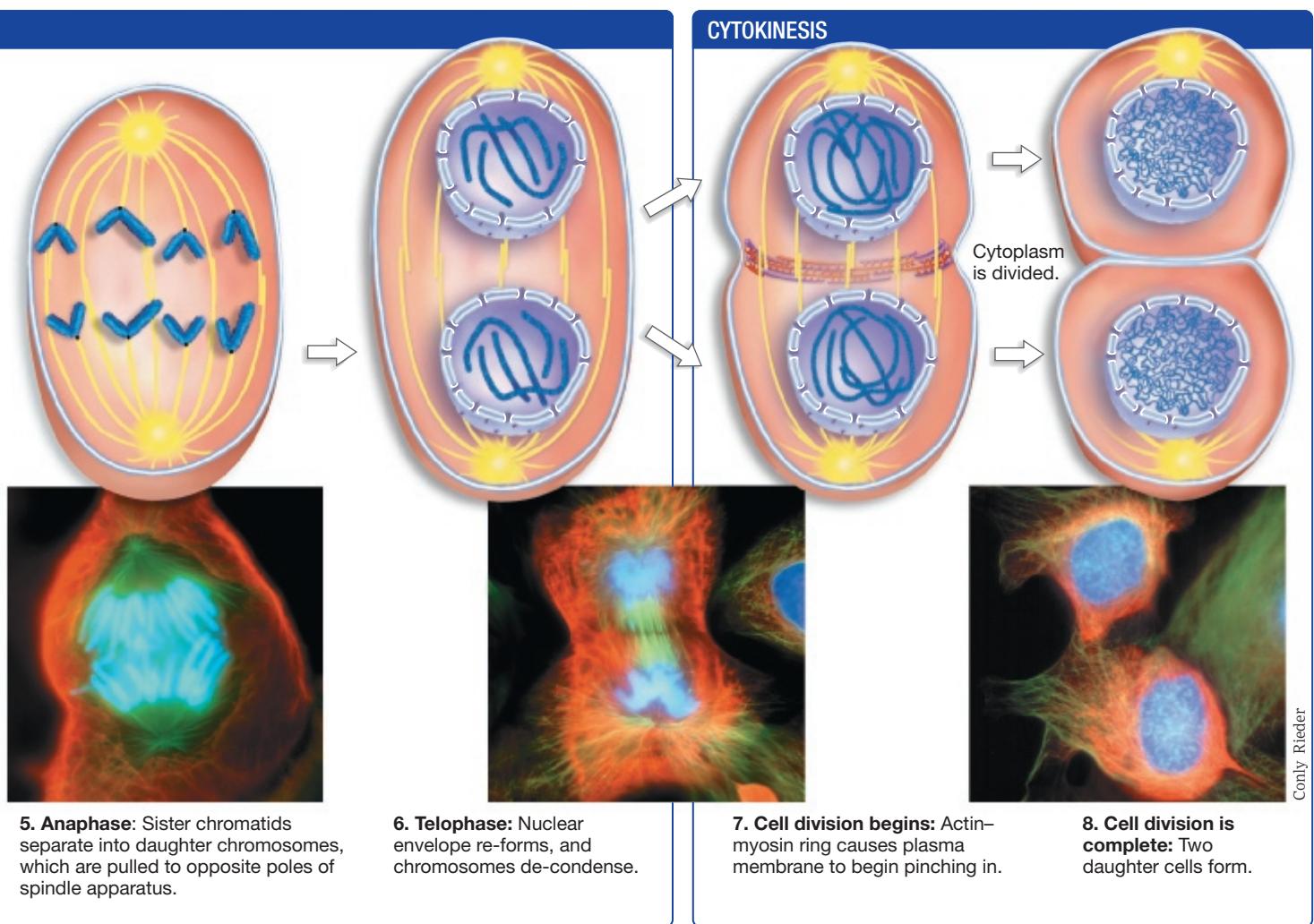
Telophase During **telophase** (“end phase”), the nuclear envelope that dissolved in prometaphase reforms around each set

of chromosomes, and the chromosomes begin to de-condense (Figure 12.5, step 6). Once two independent nuclei have formed, mitosis is complete. At this point, most cells will go on to divide their cytoplasm via cytokinesis to form two daughter cells.

Table 12.1 summarizes the key structures involved in mitosis. ▶ After you’ve studied Table 12.1 and reviewed Figure 12.5, you should be able to make a new table that summarizes what happens to (1) the spindle apparatus, (2) the nuclear envelope, and (3) the chromosomes in each of the five phases of mitosis. You should also be able to explain the purpose of each event in mitosis and what is causing it to occur.

How Do Chromosomes Move during Anaphase?

The exact and equal partitioning of genetic material to the two daughter nuclei is the most fundamental aspect of mitosis. To understand how sister chromatids separate and move to opposite sides of the spindle, biologists have focused on the role



SUMMARY Table 12.1 Structures and Proteins Involved in Mitosis

Structure	Definition
Chromosome	A structure composed of DNA and associated proteins.
Chromatin	Chromosomes within an interphase nucleus.
Sister chromatids	The two identical pieces of DNA found in a chromosome after DNA replication.
Centromere	The DNA region that functions as a handle on a chromosome.
Cohesins	Proteins at the centromeres that hold sister chromatids together.
Microtubules	Proteins that have many functions, including moving chromosomes during mitosis.
Kinetochore proteins	Proteins at the centromeres that hold the DNA and microtubules together.
Nuclear lamins	Intermediate filaments on the inner surface of the nuclear envelope that hold the nucleus together during interphase.
Condensins	Proteins along the length of chromosomes that compact them for mitosis.
Centrosomes	The microtubule-organizing centres used in animal cells during mitosis.
NEDD1 complexes	The microtubule-organizing centres used in plant cells during mitosis.

of kinetochore microtubules. How do these microtubules pull chromatids apart?

Mitotic Spindle Forces The spindle apparatus is composed of microtubules. Recall from Chapter 7 that:

- Microtubules are assembled from tubulin heterodimers. Each of these proteins is made of one α -tubulin and one β -tubulin polypeptide.
- Microtubules are asymmetric—one end is designated plus and the other is minus.
- The plus end is where microtubule growth normally occurs. Microtubule disassembly is more frequent at the minus end.

During mitosis, the microtubules originating from the spindle poles are highly dynamic. Rapid growth and disassembly ensures that some of the microtubules will be able to attach to kinetochores with their plus ends. Others will be stabilized by different proteins in the cytoplasm and become polar or astral microtubules.

These observations suggest two hypotheses for the movement of chromosomes during anaphase. The simpler hypothesis is that kinetochore microtubules stop growing at their plus ends but remain attached to the kinetochores. As the minus ends disassemble at the spindle poles, the chromosomes would be reeled in like hooked fish. An alternative hypothesis is that the chromosomes move along microtubules that are being disassembled at their plus ends at the kinetochores. In this case, each chromosome would be like a yo-yo running up a string into your hand.

To test these hypotheses, biologists at the University of Wisconsin–Madison introduced fluorescently labelled tubulin subunits into prophase or metaphase cells. This treatment made the kinetochore microtubules visible (Figure 12.6, step 1). Once anaphase began, the researchers marked a bar-shaped region of these microtubules with a beam of laser light. The laser permanently bleached sections of the fluorescently labelled microtubules, darkening them—although they were still functional (Figure 12.6, step 2).

As anaphase progressed, two things happened: (1) The darkened sections of the microtubules appeared to remain stationary, and (2) the chromosomes moved closer to the darkened sections, eventually overtaking them.

This result suggested that the kinetochore microtubules remain stationary during anaphase, but shorten because tubulin subunits are lost from their plus ends. As the microtubule ends shrink back to the spindle poles, the chromosomes are pulled along. But if the microtubule is disassembling at the kinetochore, how does the chromosome remain attached?

Kinetochores Are Linked to Retreating Microtubule Ends The kinetochore is a complex of many proteins that attaches the centromere region of the chromosome to one or more microtubules. Figure 12.7 shows a current model of kinetochore structure and function during chromosome movement in anaphase. For simplicity, a yeast kinetochore is shown, which attaches to only one microtubule. (Other eukaryotes can have as many as 30 microtubules attached to each kinetochore.)

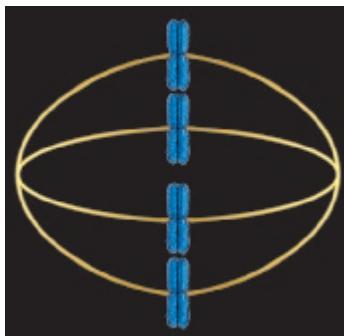
RESEARCH

QUESTION: How do kinetochore microtubules shorten to pull daughter chromosomes apart during anaphase?

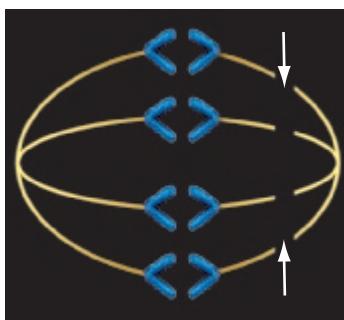
HYPOTHESIS: Microtubules shorten at the spindle pole.

ALTERNATIVE HYPOTHESIS: Microtubules shorten at the kinetochore.

EXPERIMENTAL SETUP:



1. **Label targets:** Use fluorescent labels to make the metaphase chromosomes fluoresce blue and the microtubules fluoresce yellow.

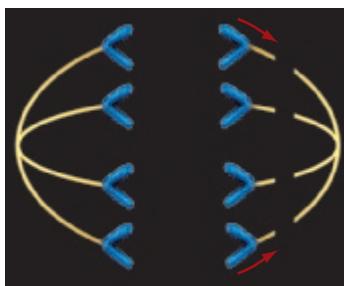


2. **Mark microtubules:** At the start of anaphase, darken sections of microtubules to mark them without changing their function.

PREDICTION:

PREDICTION OF ALTERNATIVE HYPOTHESIS: Daughter chromosomes will move toward the pole faster than the darkened sections.

RESULTS:



The darkened sections of the microtubules remained stationary as the chromosomes moved through them toward the pole.

CONCLUSION: Kinetochore microtubules shorten at the kinetochore to pull daughter chromosomes apart during anaphase.

Figure 12.6 During Anaphase, Microtubules Shorten at the Kinetochore.

SOURCE: Based on Gorbsky, G. J., P. J. Sammak, and G. G. Borisy. 1987. Chromosomes move poleward in anaphase along stationary microtubules that coordinately disassemble from their kinetochore ends. *The Journal of Cell Biology* 104: 9–18.

✓ Complete the prediction for the hypothesis that microtubules shorten at the spindle pole.

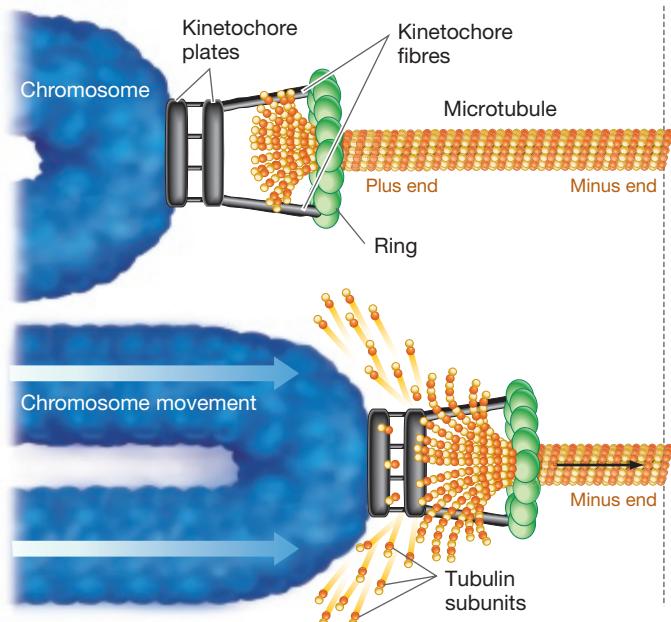


Figure 12.7 How Do Microtubules Move Chromosomes during Anaphase? Microtubules are disassembled at the kinetochore during anaphase. In yeast, kinetochore plates and fibres tether the chromosome to a ring that is pushed toward the spindle pole by the fraying plus end of the microtubule.

Fibres that extend from the yeast kinetochore are tethered to a ring that surrounds the kinetochore microtubule (Figure 12.7, top). Biologists have found that as anaphase gets under way, the plus end of the kinetochore microtubule begins to fray and

disassemble. As the fraying end widens, its expansion forces the ring, and the attached chromosome, toward the minus end of the microtubule (see Figure 12.7, bottom). The result is that the chromosome is pulled to the spindle pole by the depolymerization of the kinetochore microtubule.

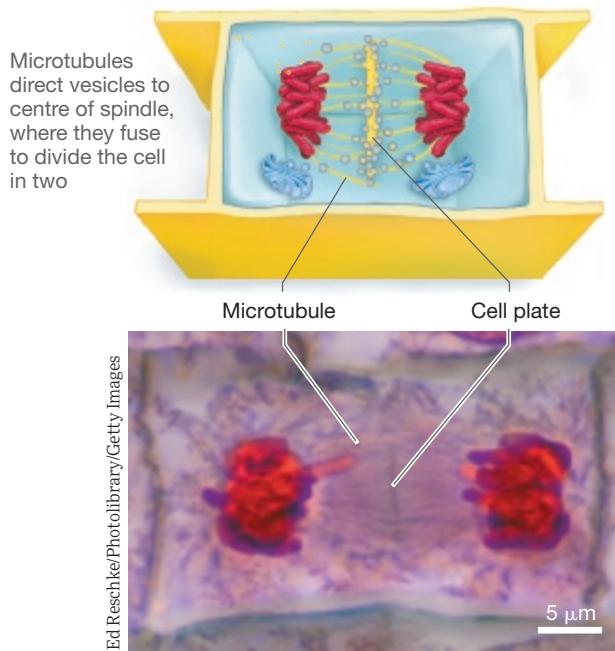
Cytokinesis Results in Two Daughter Cells

At this point, the chromosomes have been replicated in S phase and distributed to opposite sides of the spindle via mitosis. Now it's time to divide the cell into two daughters that contain identical copies of each chromosome. If these cells are to survive, however, the parent cell must also ensure that more than just chromosomes make it into each daughter cell.

While the cell was in interphase, the cytoplasmic contents, including the organelles, increased in number or volume. During cytokinesis (Figure 12.5, steps 7 and 8), the cytoplasm divides to form two daughter cells, each with its own nucleus and complete set of organelles. In most types of cells, cytokinesis directly follows mitosis.

In plants, polar microtubules left over from the spindle apparatus help define and organize the region where the new plasma membranes and cell walls will form. Vesicles from the Golgi apparatus carry components for a new cell wall to the middle of the dividing cell. These vesicles are moved along the polar microtubules via motor proteins. In the middle of what was the spindle, the vesicles start to fuse and form a flattened, sac-like structure called the **cell plate** (Figure 12.8a). The cell plate continues to grow as new vesicles fuse with it. Eventually, the cell plate contacts and fuses with the existing plasma membrane, dividing the cell into two daughter cells.

(a) Cytokinesis in plants



(b) Cytokinesis in animals

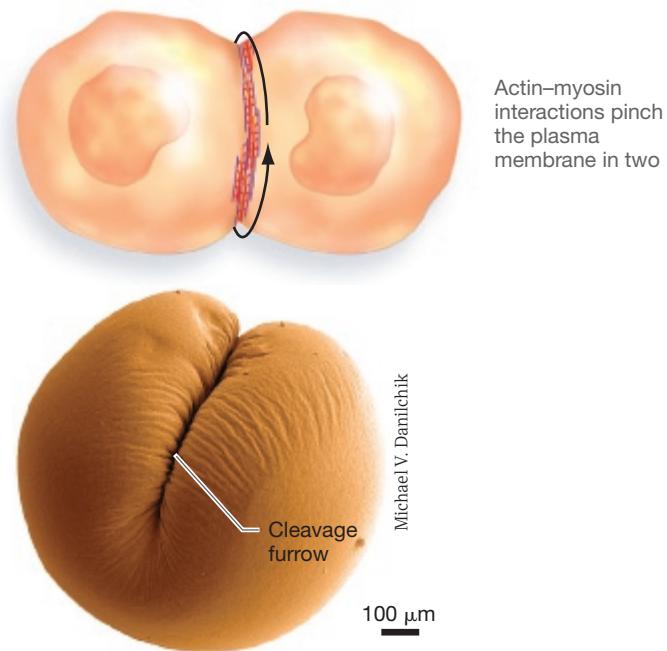


Figure 12.8 The Mechanism of Cytokinesis Varies among Eukaryotes. (a) In plants, the cytoplasm is divided by a cell plate that forms in the middle of the parent cell. (b) In animals, the cytoplasm is divided by a cleavage furrow. (The cells in both micrographs have been stained or colourized.)

In animals and many other eukaryotes, cytokinesis begins with the formation of a **cleavage furrow** (Figure 12.8b). The furrow appears when a ring of overlapping actin filaments starts to contract just inside the plasma membrane, in the middle of what used to be the spindle. This contraction is caused by myosin motor proteins that bind to the actin filaments and use adenosine triphosphate (ATP) to slide the filaments past one another (see Chapter 7).

As myosin moves the actin filaments, the ring shrinks and tightens. Because the ring is attached to the inside of the plasma membrane, the contracting ring pulls the membrane with it. As a result, the plasma membrane is drawn inward. Myosin continues to slide the actin filaments past each other, tightening the ring further, until the plasma membrane fuses and cell division is complete.

Chromosome separation and cytoplasmic division are common requirements for all organisms, not just eukaryotes. What is known about cell division in prokaryotes? Is the process of cell division in your cells similar to that in bacteria?

Bacterial Cell Replication

Many bacteria divide using a process called **binary fission**. Although binary fission does not involve mitosis, recent research has shown that chromosome segregation and cytokinesis in bacteria are strikingly similar to what occurs in the eukaryotic M phase (Figure 12.9). As the bacterial chromosome is being replicated, protein filaments attach to the copies and

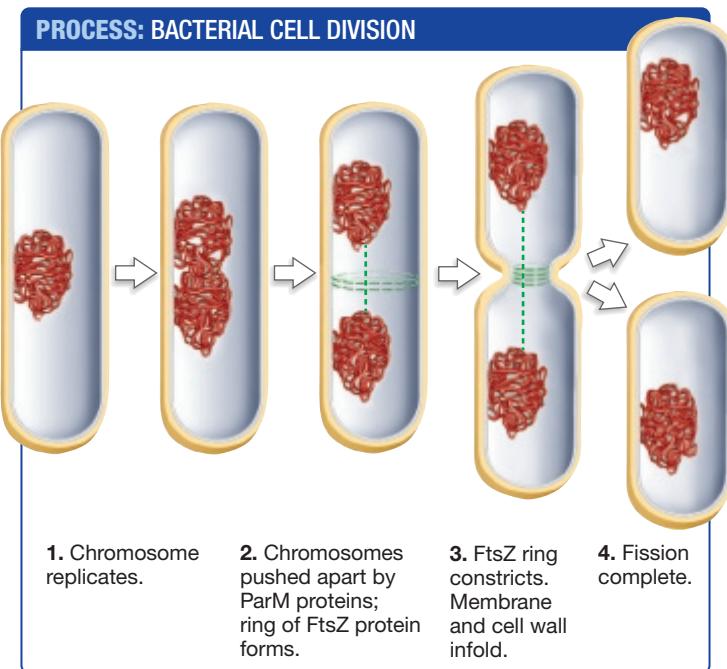


Figure 12.9 Bacterial Cells Reproduce Using DNA Replication, Partitioning, and Cytokinesis.

SOURCE: Based on Ptacin J. L., S. F. Lee, E. C. Garner, et al. 2010. A spindle-like apparatus guides bacterial chromosome segregation. *Nature Cell Biology* 12: 791–798, Fig. 5.

✓ **QUESTION** What are the animal cell counterparts of the ParM and FtsZ protein fibres and the ParS DNA regions?

separate them to opposite sides of the cell in a process called **partitioning**. The protein filaments are made of ParM monomers and the sites on the chromosome the filaments attached to are known as ParS.

Cytokinesis resembles the process as it occurs in animal cells. A ring of FtsZ proteins forms in the middle of the cell. FtsZ is one of two prokaryotic cytoskeleton proteins introduced in Canadian Research 7.1. The ring constricts, dividing the cell in two and producing two identical daughter cells.

Having explored what occurs during cell division, let's focus on how it is controlled in eukaryotes. When does a eukaryotic cell divide, and when does it stop dividing?

CHECK YOUR UNDERSTANDING

If you understand that ...

- Before a cell can reproduce, it must replicate its DNA so there are two identical copies of each gene.
- The separation of the DNA to the poles of the cell is called mitosis in eukaryotic cells and partitioning in prokaryotic cells.
- Cytokinesis occurs by different mechanisms in animal cells, plant cells, and bacterial cells but the goal is the same—separation of one cell into two cells.

✓ You should be able to ...

- Draw the mitotic spindle for an animal cell that has two chromosomes in metaphase. Label the sister chromatids, kinetochores, centrosomes, and three types of microtubules.
- Use your drawing to explain the two types of movement that are responsible for separating daughter chromosomes during anaphase.
- Compare and contrast cytokinesis in plant and animal cells.

Answers are available in Appendix A.

12.3 Control of the Cell Cycle

Although the events of mitosis are similar in all eukaryotes, control of the cell cycle often varies—even among cells in the same organism. In humans, for example, intestinal cells routinely divide twice a day to replace tissue that is lost during digestion, whereas mature nerve and muscle cells do not divide at all.

Most of these differences are due to variation in the length of G₁ phase. In rapidly dividing cells, G₁ is essentially eliminated. Most non-dividing cells, in contrast, are permanently stuck in G₁. Researchers refer to this arrested state as the G₀ state, or simply “G zero.” Nerve cells, muscle cells, and many other cell types enter G₀ once they have matured.

A cell’s division rate can also vary in response to changing conditions. For example, human liver cells normally divide about once per year. But if part of the liver is damaged or lost, the remaining cells divide every one or two days until repair is accomplished. Cells of unicellular eukaryotes, such as yeasts and some protists, divide rapidly only if the environment is rich in nutrients; otherwise, they enter G₀.

To explain these differences, biologists hypothesized that the cell cycle must be regulated in some way. Cell-cycle control is now the most prominent issue in research on cell division—partly because defects in control can lead to uncontrolled cell growth and cancer.

The Discovery of Cell-Cycle Regulatory Molecules

The first solid evidence for cell-cycle control molecules came to light in 1970. Researchers found that when they fused cells that were in different stages of the cell cycle, forming a single cell with two nuclei, one of the nuclei changed phases. For example, when a cell in M phase was fused with one in interphase, the nucleus of the interphase cell immediately initiated mitosis, even if its chromosomes had not been replicated.

To explain these results, the researchers hypothesized that the cytoplasm of M-phase cells contains a regulatory molecule that induces interphase cells to enter M phase. But cell-fusion experiments were difficult to control and didn't explain whether the nucleus or the cytoplasm was responsible for the induction. To address this issue, they turned to the South African clawed frog, *Xenopus laevis*.

As an egg of these frogs matures, it changes from a cell called an immature oocyte, which is arrested in G₂, to a mature egg that is arrested in M phase. The large size of these cells—more than 1 mm in diameter—makes them relatively easy to manipulate. Using extremely fine pipets, researchers could specifically examine the effects of the cytoplasm by removing a sample from an immature oocyte or mature egg and injecting it into an oocyte arrested in G₂.

When biologists purified cytoplasm from M-phase frog eggs and injected it into the cytoplasm of frog oocytes arrested in G₂, the oocytes immediately entered M phase (see **Figure 12.10**). But when the same experiment was done using the cytoplasm from immature oocytes, the cells remained in the G₂ phase. The researchers concluded that the cytoplasm of M-phase cells—but not the cytoplasm of interphase cells—contains a factor that drives immature oocytes into M phase to complete their maturation. This experiment was done by Yoshio Masui from the University of Toronto and Clement Markert from Yale University. **Canadian Research 12.1** tells of this and other discoveries made by Masui.

The factor that initiates M phase in oocytes was purified and is now called **M phase-promoting factor**, or **MPF**. Subsequent experiments showed that MPF induces M phase in all eukaryotes. For example, injecting M-phase cytoplasm from mammalian cells into immature frog oocytes results in egg maturation, and human MPF can trigger M phase in yeast cells.

MPF appears to be a general signal that says “Start M phase.” How does it work?

MPF Contains a Protein Kinase and a Cyclin MPF is made up of two distinct polypeptide subunits. One subunit is a protein kinase—an enzyme that catalyzes the transfer of a phosphate group from ATP to a target protein. Recall that phosphorylation may activate or inactivate the function of proteins by changing

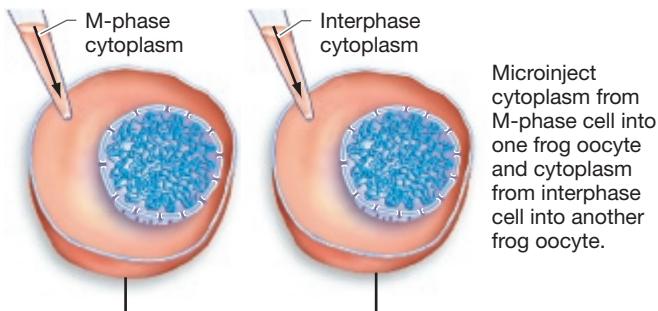
RESEARCH

QUESTION: Is M phase controlled by regulatory molecules in the cytoplasm?

HYPOTHESIS: Cytoplasmic regulatory molecules control entry into M phase.

NULL HYPOTHESIS: M-phase regulatory molecules are not in the cytoplasm or do not exist.

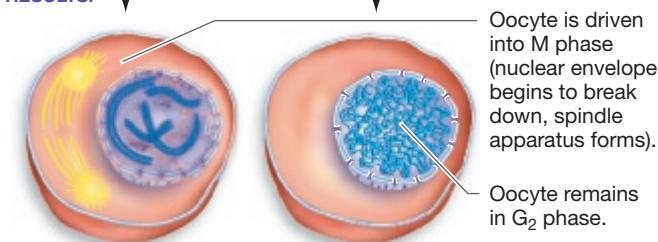
EXPERIMENTAL SETUP:



PREDICTION: Only the oocyte injected with M-phase cytoplasm will begin M phase.

PREDICTION OF NULL HYPOTHESIS: Neither oocyte will begin M phase.

RESULTS:



CONCLUSION: M-phase cytoplasm contains a regulatory molecule that induces M phase in interphase cells.

Figure 12.10 Experimental Evidence for Cell-Cycle Control Molecules. When the cytoplasm from M-phase cells is microinjected into cells in interphase, the interphase chromosomes condense, and the cells begin M phase.

SOURCE: Based on Masui, Y., and C. L. Markert. 1971. Cytoplasmic control of nuclear behavior during meiotic maturation of frog oocytes. *Journal of Experimental Zoology* 177: 129–145.

✓ How did the investigators know that it wasn't the injection itself that caused the cell on the left to enter M phase?

their shape (Chapter 8). As a result, kinases frequently act as regulatory proteins in the cell.

These observations suggested that MPF phosphorylates proteins that trigger the onset of M phase. But research showed that the concentration of the protein kinase is more or less constant throughout the cell cycle. How can MPF trigger M phase if the protein kinase subunit is always present?

The answer lies in the second MPF subunit, which belongs to a family of proteins called **cyclins**. Cyclins got their name because their concentrations fluctuate throughout the cell cycle.

CANADIAN RESEARCH 12.1 Yoshio Masui and the Discovery of MPF

The experiment shown in Figure 12.10 was done in part by Yoshio Masui (**Figure 12.11**) from the University of Toronto. These cytoplasm injection experiments demonstrated that a cell's entry into mitosis was controlled by an unknown factor in the cytosol, which the researchers named the mitosis-promoting factor (MPF). The more M-phase cytosol that was injected into the frog oocytes, the more likely the oocytes were to enter mitosis.

The idea that something in the cytosol could control events in the nucleus was controversial. Was it not supposed to be that the genes in the nucleus controlled all aspects of the cell? It became important to find out what the MPF was. William Wasserman and Masui repeated the injection experiments, but this time they treated the M-phase cytosol to see what conditions would eliminate its MPF activity (**Figure 12.12**). For example, they treated it with proteases to destroy all the proteins and with ribonucleases to break down all the RNA molecules. This would allow them to determine if the MPF was made of protein and/or RNA. This experiment was similar to a famous one done 30 years before by Oswald Avery in his work with DNA (see Chapter 15). As can be seen in this experiment figure, they were successful in demonstrating that the MPF was made of one or more proteins.



Figure 12.11 Yoshio Masui.

As **Figure 12.13** shows, the concentration of the cyclin associated with MPF builds during interphase and peaks in M phase. The timing of this increase is important because the protein kinase subunit in MPF is functional only when it is bound to the cyclin subunit. As a result, the protein kinase subunit of MPF is called a **cyclin-dependent kinase**, or Cdk.

To summarize, MPF is a dimer consisting of a cyclin and a cyclin-dependent kinase. The cyclin subunit regulates the

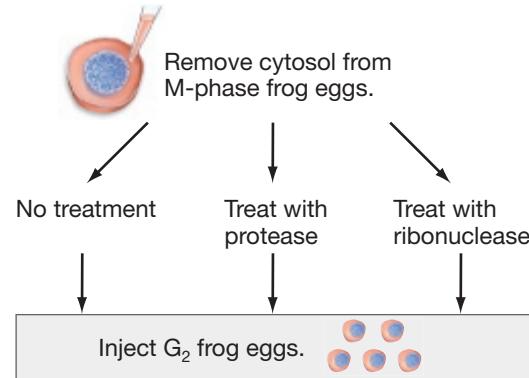
RESEARCH

Question: What does the MPF consist of?

HYPOTHESIS 1: The MPF is at least partially made of protein.

HYPOTHESIS 2: The MPF is at least partially made of RNA.

EXPERIMENTAL SETUP:



PREDICTION: At least one of these treatments should reduce or eliminate MPF activity.

RESULTS:

↓	15/15 eggs entered mitosis	↓	1/15 eggs entered mitosis	↓	15/15 eggs entered mitosis
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CONCLUSION: The MPF contains one or more proteins but no RNA.

Figure 12.12 The Experiment Done by Masui and Wasserman.

It was work by Masui and others using amphibian eggs that revealed how all eukaryotic cells regulate their cell cycles. Masui was also involved in the discovery and characterization of a protein with the opposite effect. Whereas the MPF promotes continuation of the cell cycle, the cytostatic factor (CSF) allows oocytes to pause their cell cycle while they await fertilization. Masui has been recognized for his contributions to cell biology with international scientific awards and has been made an officer of the Order of Canada.

SOURCES : Masui, Y., and C. L. Markert. 1971. Cytoplasmic control of nuclear behavior during meiotic maturation of frog oocytes. *Journal of Experimental Zoology* 177: 129–146. Also, Wasserman, W., and Y. Masui. 1976. A cytoplasmic factor promoting oocyte maturation: Its extraction and preliminary characterization. *Science* 191: 1266–1268.

Think About It: Would Masui and Markert have been able to make the same conclusion if they had done only the injection of M-phase cytoplasm treated with protease?

formation of the MPF dimer; the kinase subunit catalyzes the phosphorylation of other proteins to start M phase.

How Is MPF Turned On? According to Figure 12.13, the concentration of cyclin builds up steadily during interphase. Why doesn't the resulting increase in the concentration of MPF trigger the onset of M phase earlier in the cell cycle?

The answer is that the activity of MPF's Cdk subunit is further regulated by two phosphorylation sites on the subunit.

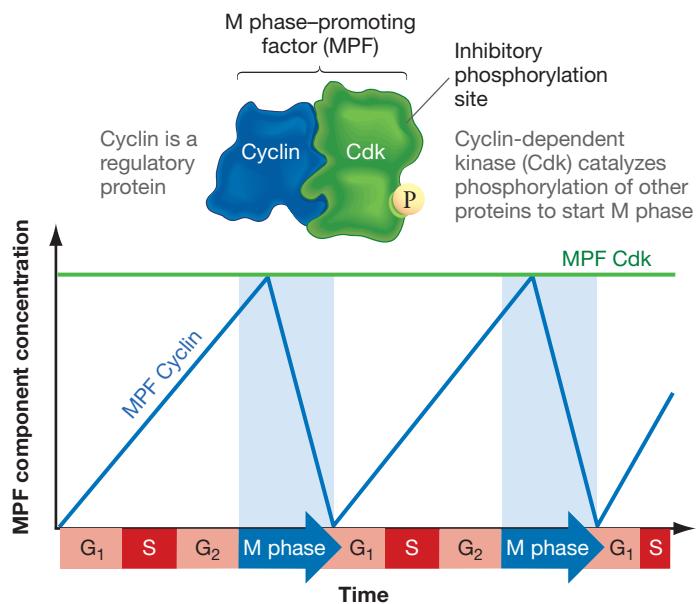


Figure 12.13 Cyclin Concentration Regulates the Concentration of the MPF Dimer. Cyclin concentrations fluctuate in dividing cells, reaching a peak in M phase. The activity of MPF, shown in the blue shaded areas, requires both cyclin and Cdk components.

✓ In this figure, the concentration of cyclin declines rapidly during M phase. Why do you think this decline is important?

Phosphorylation of one site activates the kinase, but phosphorylation of the second site inhibits the kinase. Both sites are phosphorylated after cyclin binds to the Cdk subunit. This allows the concentration of the dimer to increase without prematurely starting M phase. Late in G₂ phase, however, an enzyme removes the inhibitory phosphate. This dephosphorylation reaction, coupled with the addition of the activating phosphate, changes the Cdk's shape in a way that turns on its kinase activity.

What Does MPF Do? Once MPF is activated, it triggers several events that shift the cell from interphase into mitosis. These include (1) the reorganization of microtubules into a mitotic spindle, (2) the dismantlement of the nuclear envelope, and (3) chromosome condensation.

To bring about the first two events, MPF phosphorylates microtubule regulators and various components of the nuclear envelope, respectively. But what of chromosome condensation? Could MPF be phosphorylating and activating condensins directly? Recent research, discussed in **Canadian Research 12.2**, confirms this simple hypothesis.

How Is MPF Turned Off? During anaphase, an enzyme complex begins degrading MPF's cyclin subunit, triggering a chain of events that leads to the deactivation of MPF.

MPF deactivation illustrates two key concepts about regulatory systems in cells:

- **Negative feedback** occurs when a process is slowed or shut down by one of its products. Thermostats shut down furnaces

CANADIAN RESEARCH 12.2 MPF Activates Condensins Directly

At the start of prophase, MPF is activated and the chromosomes condense. Does one event cause the other or are these separate events that coincide? And if MPF does cause chromosome condensation, does it do so directly or indirectly? Researchers at the Université de Montréal and McGill University did a series of experiments to find out. They used *Saccharomyces cerevisiae* because this single-celled yeast is well suited to scientific investigations (see **BioSkills 9**).

Their results supported a three-part model: (1) MPF phosphorylates condensin proteins at the start of mitosis, (2) phosphorylated

condensins have an increased affinity for DNA, (3) condensins bind to DNA and condense it. **Table 12.2** shows their experiments, results, and conclusions.

Think About It: Add the conclusions for Experiments 2 to 5 in the table below.

SOURCE: Based on Robelett, X. et al. 2015. A high-sensitivity phoso-switch triggered by Cdk1 governs chromosome morphogenesis during cell division. *Genes & Development* 29: 426–439.

Table 12.2 Experiments That Revealed What Causes Chromosomes to Condense in Prophase.

Experiment	Result	Conclusion
1. They made cells with defective MPF proteins.	The cells were unable to condense their chromosomes.	MPF is necessary, either directly or indirectly, for chromosome condensation.
2. They made cells with condensins that had been altered so that they could not be phosphorylated.	The cells were unable to condense their chromosomes.	
3. They mixed purified condensins, purified MPFs, and ATP in a test tube.	The condensins were phosphorylated.	
4. They isolated condensins from cells at different stages of the cell cycle.	Condensins from G ₁ cells were not phosphorylated; condensins from cells entering mitosis were phosphorylated.	
5. They made cells with condensins that had been altered so that they could not be phosphorylated.	The altered condensins had a lower affinity for DNA.	

when temperatures are high; enzymes in glycolysis are inhibited by ATP (see Chapter 9); MPF is turned off by an enzyme complex that is activated by events in mitosis.

- Destroying specific proteins is a common way to control cell processes. In the case of MPF, the enzyme complex that is activated in anaphase attaches small proteins called ubiquitins to MPF's cyclin subunit. This marks the subunit for destruction by a protein complex known as the proteasome.

In response to MPF activity, then, the concentration of cyclin declines rapidly. It slowly builds up again during interphase.

- ◆ If you understand this aspect of cell-cycle regulation, you should be able to explain the relationship between MPF and cyclin, Cdk, and the enzymes that phosphorylate MPF, dephosphorylate MPF, and degrade cyclin.

Cell-Cycle Checkpoints Can Arrest the Cell Cycle

The dramatic changes in cyclin concentration and Cdk activity drive the ordered events of the cell cycle. These events are occurring in your body right now. Over a 24-hour period, you swallow millions of cheek cells and lose millions of cells from your intestinal lining as waste. To replace them, other cells in your cheek and intestinal tissue are making and degrading cyclin and pushing themselves through the cell cycle.

MPF is only one of many protein complexes involved in regulating the cell cycle, however. A different cyclin complex triggers the passage from G₁ phase into S phase. Because it is made of a G₁-cyclin working with a cyclin-dependent kinase, it is called **G₁-Cdk**. There are several other regulatory molecules that can hold cells in particular stages. Two American scientists, Leland Hartwell and Ted Weinert, untangled all these incongruous regulatory systems.

To make sense of these observations, they introduced the concept of **cell-cycle checkpoints**. A cell-cycle checkpoint is a critical point in the cell cycle that is regulated.

Hartwell and Weinert identified checkpoints by analyzing yeast cells with defects in the cell cycle. The defective cells kept dividing under culture conditions that caused normal cells to stop dividing, because the defective cells lacked a specific checkpoint. In multicellular organisms, cells that keep dividing in this way may form a mass of cells called a **tumour**.

There are distinct checkpoints in three of the four phases of the cell cycle (Figure 12.14). In effect, interactions among regulatory molecules at each checkpoint allow a cell to “decide” whether to proceed with division or not. If these regulatory molecules are defective, the checkpoint may fail and cells may start dividing in an uncontrolled fashion.

G₁ Checkpoint The first cell-cycle checkpoint occurs late in G₁ phase. For most cells, this checkpoint is the most important in establishing whether the cell will continue through the cycle and divide, or exit the cycle and enter G₀. What factors are important in determining whether a cell passes the G₁ checkpoint?

- **Size** Because a cell must reach a certain size before its daughter cells will be large enough to function normally, biologists hypothesize that some mechanism exists to arrest the cell cycle if the cell is too small.

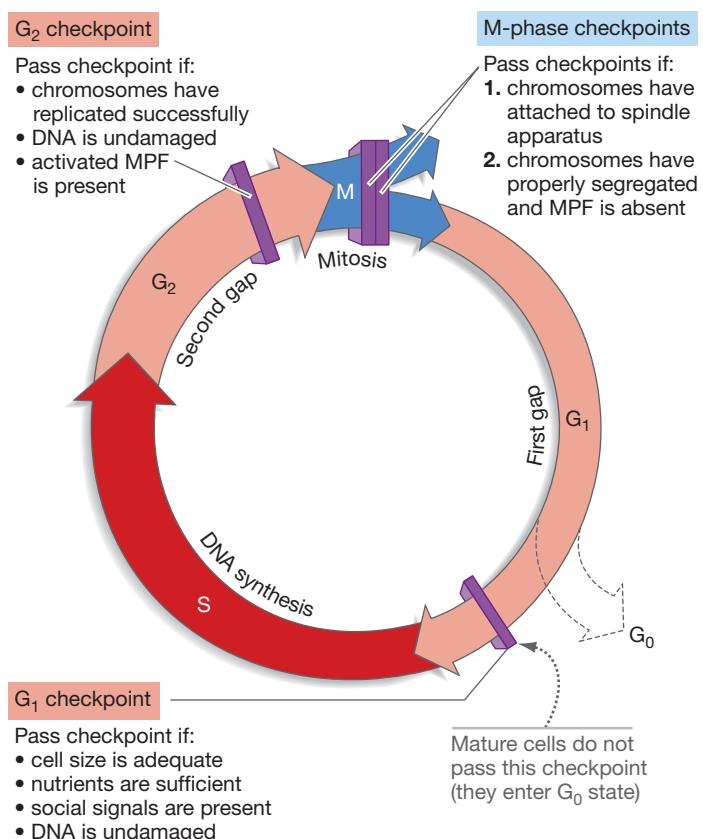


Figure 12.14 The Four Cell-Cycle Checkpoints.

- **Availability of nutrients** Unicellular organisms arrest at the G₁ checkpoint if nutrient conditions are poor.
- **Social signals** Cells in multicellular organisms pass (or do not pass) the G₁ checkpoint in response to signalling molecules from other cells, which are termed social signals.
- **Damage to DNA** If DNA is physically damaged, the protein p53 activates genes that either stop the cell cycle until the damage can be repaired or cause the cell's programmed, controlled destruction—a phenomenon known as **apoptosis**. In this way, p53 acts as a brake on the cell cycle.

If “brake” molecules such as p53 are defective, damaged DNA remains unrepaired. Damage in genes that regulate cell growth can lead to uncontrolled cell division. Consequently, regulatory proteins like p53 are called **tumour suppressors**.

G₂ Checkpoint The second checkpoint occurs after S phase, at the boundary between the G₂ and M phases. Because MPF is the key signal triggering the onset of M phase, investigators were not surprised to find that it is involved in the G₂ checkpoint.

Data suggest that if DNA is damaged or if chromosomes are not replicated correctly, the inhibitory phosphate on MPF's Cdk subunit is not removed. As a result, MPF is not turned on, and cells remain in G₂ phase. Cells at the G₂ checkpoint may also respond to signals from other cells and to internal signals relating to cell size.

M-Phase Checkpoints The final two checkpoints occur during mitosis. The first regulates the transition from metaphase to anaphase. This checkpoint ensures that the sister chromatids do not

split until all kinetochores are attached properly to the spindle apparatus. If the metaphase checkpoint did not exist, some chromosomes might not separate correctly, and daughter cells would receive either too many or too few chromosomes.

The second checkpoint regulates the transition from anaphase to telophase. To exit M phase and progress into G₁ phase, cells must degrade all of their cyclins and thus turn off MPF activity. The enzymes responsible for degrading cyclins are activated only when all the chromosomes have been properly separated. If chromosomes do not fully separate during anaphase, the remaining MPF activity will prevent the cell from entering telophase and undergoing cytokinesis. If cells are arrested by either of these two checkpoints, they will remain in M phase.

To summarize, the four cell-cycle checkpoints have the same purpose: They prevent the division of cells that are damaged or that have other problems. The G₁ checkpoint also prevents mature cells that are in the G₀ state from dividing.

Understanding cell-cycle regulation is fundamental. If one of the checkpoints fails, the affected cells may begin dividing in an uncontrolled fashion. For a multicellular organism as a whole, the consequences of uncontrolled cell division may be dire: cancer.

CHECK YOUR UNDERSTANDING

If you understand that ...

- The cell cycle consists of four carefully controlled phases.

✓ You should be able to ...

List where the four cell-cycle checkpoints occur in the cell cycle and explain why they are important.

Answers are available in Appendix A.

12.4 Cancer: Out-of-Control Cell Division

Forty-five percent of Canadian men and forty-two percent of Canadian women will develop cancer during their lifetime. In Canada, one in four of all deaths is from cancer. In 2011, Statistics Canada reported that cancer now exceeds heart disease as the leading cause of death among Canadians.

Cancer is a general term for disease caused by cells that divide in an uncontrolled fashion, invade nearby tissues, and spread to other sites in the body. Cancerous cells cause disease because they use nutrients and space needed by normal cells and disrupt the function of normal tissues.

Humans suffer from at least 200 types of cancer. Stated another way, cancer is not a single illness but a complex family of diseases that affect an array of organs, including the breast, colon, brain, lung, and skin. In addition, several types of cancer can affect the same organ. Skin cancers, for example, come in multiple forms.

Some cancers are relatively easy to treat; others are often fatal. **Figure 12.15** illustrates how mortality rates due to different types of cancer have changed through time in the United States. The pattern in Canada is similar. The most recent Canadian statistics can be found at www.cancer.ca.

Let's review the general characteristics of cancer and then explore how regulatory mechanisms become defective.

Properties of Cancer Cells

When even a single cell in a multicellular organism begins to divide in an uncontrolled fashion, a mass of cells called a tumour may result. Some tumours can be surgically removed without damage to the affected organ. Often, though, tumour removal doesn't cure cancer. Why?

In addition to uncontrolled replication, cancer cells are invasive—meaning that they are able to spread to adjacent tissues and throughout the body via the bloodstream or the lymphatic vessels (introduced in Chapter 42), which collect excess fluid from tissues and return it to the bloodstream.

Invasiveness is a defining feature of a **malignant tumour**—one that is cancerous. Masses of noninvasive cells are noncancerous and form **benign tumours**. Some benign tumours are largely harmless. Others grow quickly and can cause problems if they are located in the brain or other sensitive parts of the body.

Cells in a tumour become cancerous if they gain the ability to detach from the tumour and invade other tissues. By spreading from the primary tumour site, cancer cells can establish secondary tumours elsewhere in the body (**Figure 12.16**). This process is called **metastasis**.

If metastasis has occurred by the time the original tumour is detected, secondary tumours may have already formed and surgical removal of the primary tumour will not lead to a cure. This is why early detection is the key to treating cancer most effectively.

Causes of Cancer

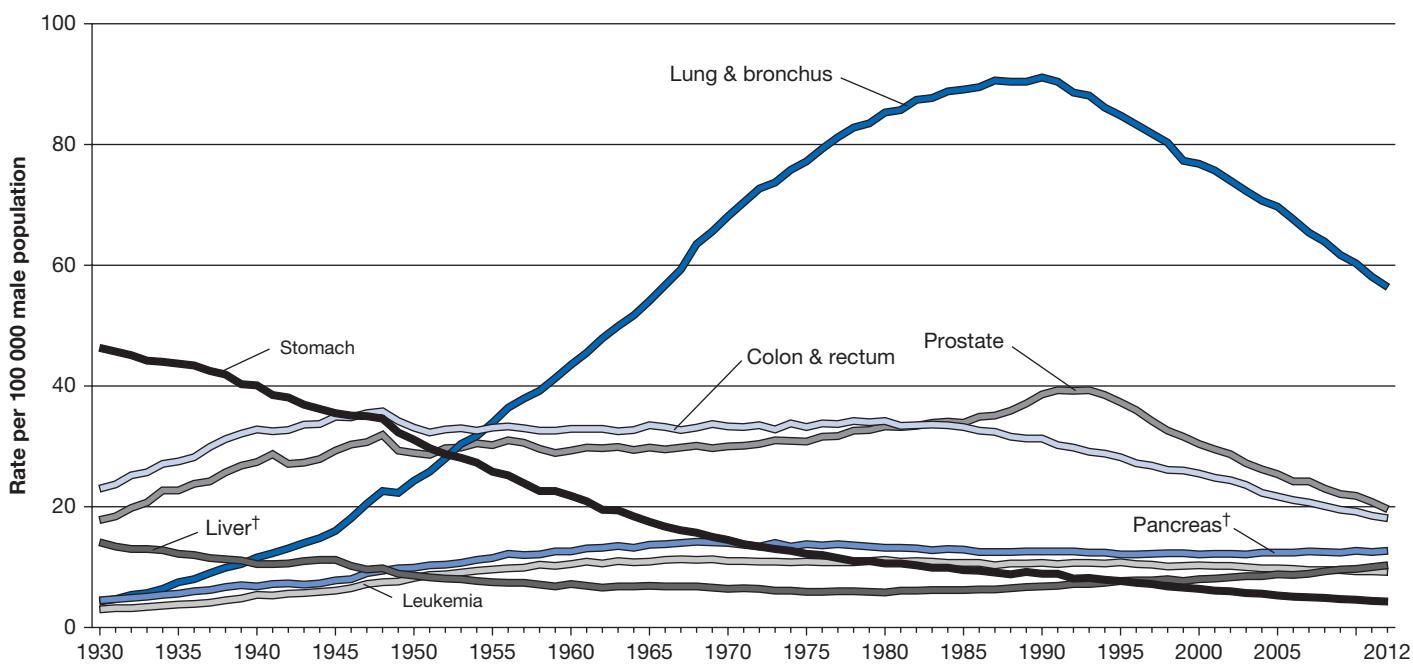
What causes cancer at the molecular level? Essentially it is due to cells ignoring the rules that govern how they are supposed to behave in a multicellular organism.

Loss of Social Control In unicellular eukaryotes, passage through the G₁ checkpoint is thought to depend primarily on cell size and the availability of nutrients. If nutrients are plentiful, cells grow, pass through the checkpoint, and divide rapidly.

In multicellular organisms, however, cells divide in response to signals from other cells. Biologists refer to this as **social control** over cell division. The general idea is that individual cells are allowed to divide only when it is in the best interests of the organism as a whole.

Social control of the cell cycle is based on **growth factors**—polypeptides or small proteins that stimulate cell division. Many growth factors were discovered by researchers who were trying to grow cells in culture. When isolated mammalian cells were placed in a culture flask and provided with adequate nutrients, they arrested in G₁ phase. The cells began to grow again only when biologists added **serum**—the liquid portion of blood that remains after blood cells and cell fragments have been removed. Researchers identified growth factors as the components in the serum that were responsible for allowing cells to pass through the G₁ checkpoint.

Growth factors are molecules that travel from cell to cell. When they arrive at a target cell, they bind to growth factor receptors on the surface. The receptor proteins send a signal into the cell that activates the G₁-Cdk proteins. The cell then moves beyond the G₁ checkpoint and prepares for S phase.

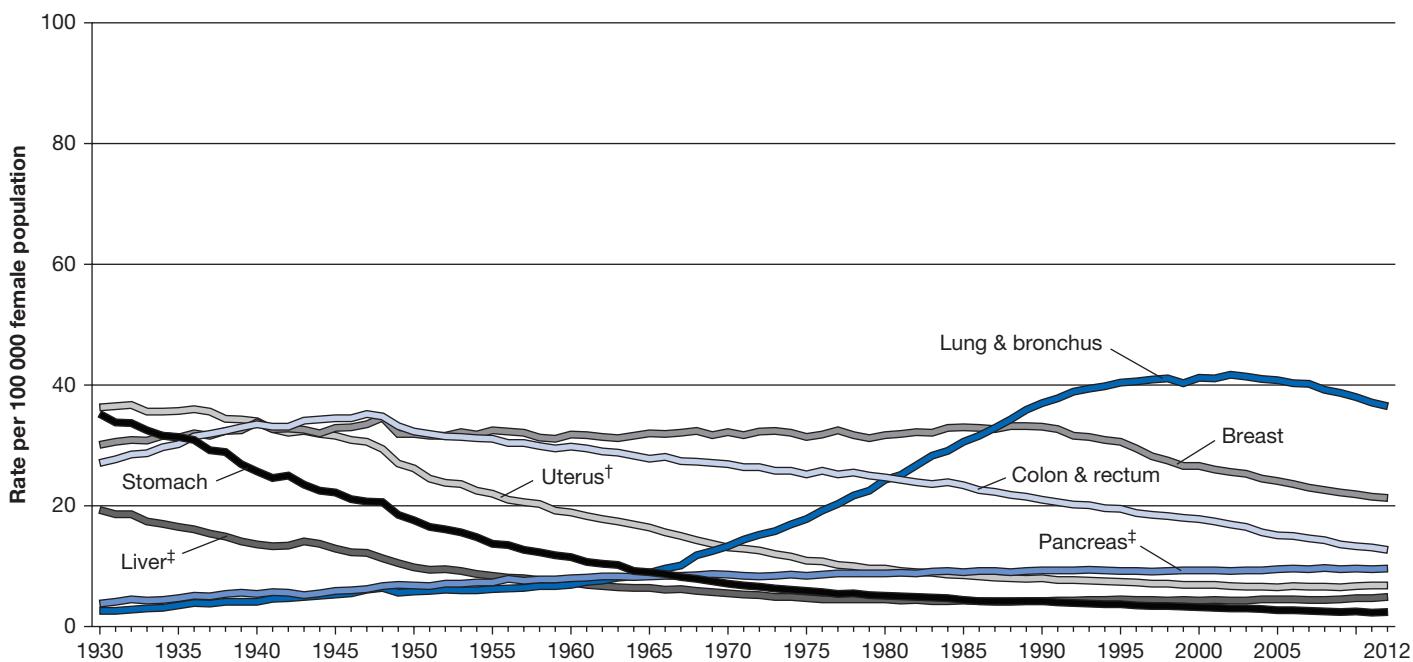


*Per 100,000, age adjusted to the 2000 US standard population. †Mortality rates for pancreatic and liver cancers are increasing.

NOTE: Due to changes in ICD coding, numerator information has changed over time. Rates for cancers of the liver, lung and bronchus, and colon and rectum are affected by these coding changes.

SOURCE: US Mortality Volumes 1930 to 1959 and US Mortality Data 1960 to 2012, National Center for Health Statistics, Centers for Disease Control and Prevention.

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*Per 100,000, age adjusted to the 2000 US standard population. †Uterus refers to uterine cervix and uterine corpus combined. ‡Mortality rates for pancreatic and liver cancers are increasing.

NOTE: Due to changes in ICD coding, numerator information has changed over time. Rates for cancers of the liver, lung and bronchus, and colon and rectum are affected by these coding changes.

SOURCE: US Mortality Volumes 1930 to 1959, US Mortality Data 1960 to 2012, National Center for Health Statistics, Centers for Disease Control and Prevention.

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Figure 12.15 Cancer Death Rates in the United States. These rates vary over time because of changes in incidence (how often people get a particular cancer), detection, and treatment success.

SOURCE: Based on data from the website of the National Cancer Institute (<http://www.cancer.gov>), Common Cancer Statistics, December 2014.

- ✓ How has the death rate due to lung cancer changed over time in males versus females? Suggest a hypothesis to explain this pattern.

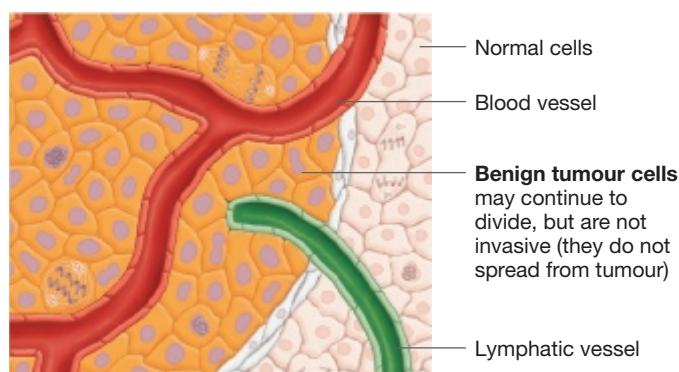
✓ If you understand what growth factors do, you should be able to explain how having too many growth factor receptors on a cell can lead to that cell becoming cancerous.

Loss of p53 Proteins About 85 percent of lung cancers are caused by cigarette smoking. The actual mechanism for this was discovered by a group of American scientists in 1996. Moonshong Tang, Gerd Pfeifer, and two colleagues suspected that the p53 protein was involved. Recall from earlier in this chapter that this protein limits cell replication.

When they combined their research with those of others, two conclusions emerged:

1. **Smokers with lung cancer had mutations in the gene that makes p53 proteins.** Scientists had sequenced the DNA from the p53 gene in more than 500 smokers with lung

(a) Benign tumour



(b) Malignant tumour

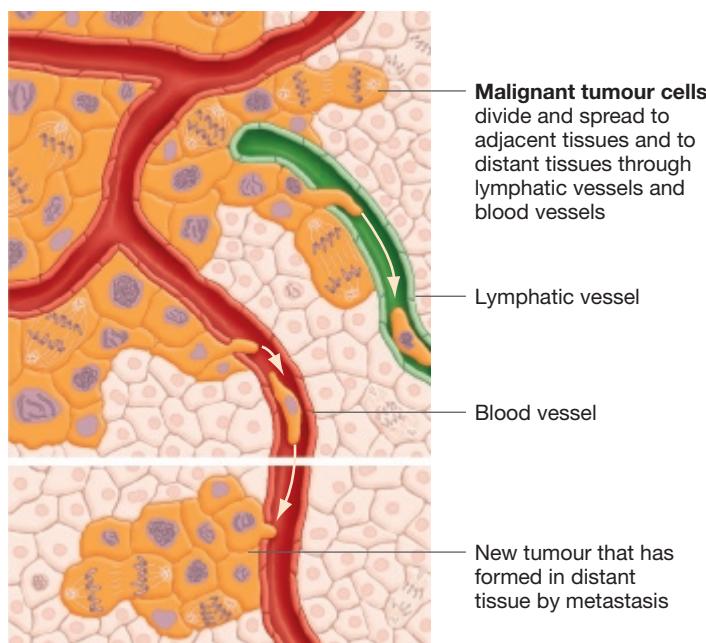


Figure 12.16 Cancers Spread to New Locations in the Body.

(a) Benign tumours grow in a single location. (b) Malignant tumours are invasive and may be metastatic—meaning that their cells can spread to distant parts of the body and initiate new tumours. Malignant tumours cause cancer.

cancer. They found that three places in the gene were frequently mutated.

2. **A chemical in cigarette smoke causes mutations in the p53 gene.** Cigarette smoke contains many DNA-damaging chemicals. When Tang and Pfeifer's group exposed human cells to one of them, they found that it stuck to the p53 gene. Not only that, it stuck to the same three places where the patients frequently had mutations.

Because of their findings, we know more about how cigarette smoking kills people. A person inhales cigarette smoke into their lungs, one of the chemicals it contains enters a lung cell and mutates the p53 gene, and the cell is no longer able to regulate cell replication and begins to divide uncontrollably.

In order for the lung cancer cells to become very destructive, they have to spread by metastasis (see Figure 12.16). Normally, human cells are contained within a network of collagen and other external proteins. How cancer cells cause this network to be dismantled is still poorly understood.

More than half of all cancers are due, at least in part, to loss of p53. This one protein is the subject of research across Canada and around the world.

Suppression of the Apoptosis Pathway Earlier in this chapter, you learned that p53 can activate a cellular process called apoptosis—programmed cell death. Cells that become damaged are programmed to enter this process, and most of them do. Cancer results when a cell that should die doesn't.

In 2007, scientists at the University of Alberta discovered one way this can occur. A team led by Evangelos Michelakis found that certain cancer cells had inactivated their mitochondria. Mitochondria are the organelles that synthesize ATP (see Chapter 7), but they perform other functions in the cell, including triggering apoptosis. The scientists were testing a small synthetic chemical called dichloroacetate, or DCA (Figure 12.17), which was known to increase mitochondrial activity. When they added DCA to cancer cells growing in a culture, the cells reactivated their mitochondria, entered apoptosis, and then died. DCA was also successful at limiting the growth of tumours in rats with cancer.

Since this initial discovery, researchers at the University of Alberta and worldwide have been administering DCA to patients with cancer. Ideally, it would reactivate the mitochondria in their cancer cells, which would cause the cells to die. So far some studies have shown small benefits and others no benefits. It is too early to tell if DCA will become a “miracle drug” or will just remain a molecule that led to a better understanding of the biology of cancer cells.

DCA is easy to synthesize, which has led to a grey market for it. Some people with cancer buy DCA online and self-administer it. This is problematic because at high doses DCA causes short-term (fatigue and vomiting) and long-term (liver and nerve) damage. DCA sold online is also insufficiently pure. Often it is industrial grade rather than pharmaceutical grade. That said, it

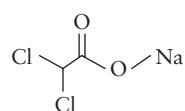


Figure 12.17 Dichloroacetate (DCA), a Potential Cancer-Treating Drug.

is not hard to see why desperate patients and their families might be willing to disregard these concerns.

The Canadian Cancer Society published a report on DCA in 2013 to address the public's perception of DCA. They summarized the lack of knowledge about its risks and benefits and concluded "...the Society does not advise cancer patients to use DCA, unless they are part of a clinical trial."¹

¹ See www.cancer.ca/en/about-us/news/national/2013/canadian-cancer-societys-perspective-on-dea/.

Because cancer is a family of diseases with a complex and highly variable molecular basis, there will be no "magic bullet," or single therapy, that cures all forms of the illness. Still, recent progress in understanding the cell cycle and the molecular basis of cancer has been dramatic, and cancer prevention and early detection programs are increasingly effective. The prognosis for many cancer patients is remarkably better now than it was even a few years ago. Thanks to research, almost all of us know someone who is a cancer survivor.

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CHAPTER 12 REVIEW

12.1 How Do Cells Replicate?

- For a cell to replicate, it must copy its chromosomes, separate the copies, and divide the cytoplasm to generate daughter cells that have the same chromosomal complement as the parent cell.
- Eukaryotic cells divide by cycling between interphase and M phase.
- Interphase consists of S phase, when chromosomes replicate, and the G₁ and G₂ phases, when cells grow and prepare for division.
- M phase consists of mitosis or meiosis, when chromosomes separate, and cytokinesis, when the parent cell divides into two daughter cells.

12.2 What Happens during M Phase?

- Mitosis can be described as a sequence of five phases:
 - Prophase** Chromosomes condense. The spindle apparatus begins to form, and polar microtubules overlap each other.
 - Prometaphase** In cells of many organisms, the nuclear envelope pulls back from the chromosomes. Microtubules attach to the kinetochores of chromosomes, which begin moving to the middle of the spindle.
 - Metaphase** All the chromosomes are positioned in the middle of the spindle. The spindle is anchored to the plasma membrane by astral microtubules.
 - Anaphase** Sister chromatids are pulled apart by the disassembly of kinetochore microtubules at the kinetochore. The separated chromatids are now daughter chromosomes. The spindle poles are moved farther apart to fully separate the replicated chromosomes.
 - Telophase** Daughter chromosomes are fully separated and are clustered at opposite poles of the spindle. A nuclear envelope forms around each set, and the chromosomes de-condense.
- In most cells, mitosis is followed by cytokinesis—division of the cytoplasm to form two daughter cells.

12.3 Control of the Cell Cycle

- The onset of the S and M phases is primarily determined by the activity of protein complexes consisting of a cyclin and a cyclin-dependent kinase (Cdk).
- Cyclin concentrations oscillate during the cell cycle, regulating the formation of the complexes. The activity of the Cdk is further regulated by addition of a phosphate in its activating site and removal of one from its inhibitory site.

- Progression through the cell cycle is controlled by checkpoints in three phases:
 - The G₁ checkpoint regulates progress based on nutrient availability, cell size, DNA damage, and social signals.
 - The G₂ checkpoint delays progress until chromosome replication is complete and any damaged DNA present is repaired.
 - The two M-phase checkpoints (1) delay anaphase until all chromosomes are correctly attached to the spindle apparatus and (2) delay the onset of cytokinesis and G₁ until all chromosomes have been properly partitioned.

12.4 Cancer: Out-of-Control Cell Division

- Cancer is characterized by (1) loss of control at the G₁ checkpoint, resulting in cells that divide in an uncontrolled fashion; and (2) metastasis, or the ability of tumour cells to spread throughout the body.
- Cancer occurs when one or more regulatory processes are damaged in a cell. These include a loss of social control, loss of p53 proteins, and suppression of the apoptosis pathway.

Answers are available in Appendix A

✓ TEST YOUR KNOWLEDGE

- Which statement about the daughter cells following mitosis and cytokinesis is correct?
 - They are genetically different from each other and from the parent cell.
 - They are genetically identical to each other and to the parent cell.
 - They are genetically identical to each other but different from the parent cell.
 - Only one of the two daughter cells is genetically identical to the parent cell.
- After S phase, what comprises a single chromosome?
 - two daughter chromosomes
 - a double-stranded DNA molecule
 - two single-stranded DNA molecules
 - two sister chromatids
- Progression through the cell cycle is regulated by oscillations in the concentration of which type of molecule?
 - p53
 - condensins
 - cyclins
 - cyclin-dependent kinases
- What major events occur during anaphase of mitosis?

✓ TEST YOUR UNDERSTANDING

5. Identify at least two events in the cell cycle that must be completed successfully for daughter cells to share an identical complement of chromosomes.
6. What evidence suggests that during anaphase, kinetochore microtubules shorten at the kinetochore?
7. Why are cohesin proteins put onto DNA *before* DNA replication and not during or after?
8. In multicellular organisms, non-dividing cells stay in G₀ phase. For the cell, why is it better to be held in G₁ rather than S, G₂, or M phase?
 - a. G₁ cells are larger and more likely to perform the normal functions of the cell.
 - b. G₁ cells have not replicated their DNA in preparation for division.
 - c. G₁ cells are the only ones that do not have their chromatin in a highly condensed state.
 - d. MPF is required to enter S phase, so the cell is committed to entering M phase if the cycle moves beyond G₁.

✓ TEST YOUR PROBLEM-SOLVING SKILLS

9. **QUANTITATIVE** A particular cell type spends 4 hours in G₁ phase, 2 hours in S phase, 2 hours in G₂ phase, and 30 minutes in M phase. If a pulse–chase experiment were performed with radioactive thymidine on an asynchronous culture of such cells, what percentage of mitotic cells would be radioactive 9 hours after the pulse?
 - a. 0 percent
 - b. 50 percent
 - c. 75 percent
 - d. 100 percent
10. When a fruit fly embryo first begins to develop, a large cell is generated that contains over 8000 genetically identical nuclei. What is most likely responsible for this result?

✓ PUT IT ALL TOGETHER: Case Study



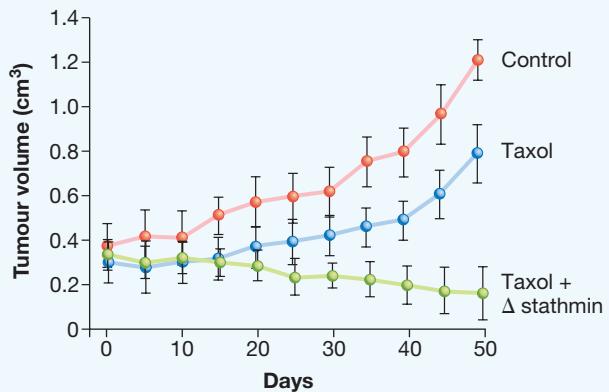
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What are the molecular targets of anticancer drugs?

The bark of the Pacific yew tree (*Taxus brevifolia*) was the original source of one of the most effective drugs for treating tumours of the breast, lung, and other sites. Taxol, a chemical extracted

from this bark, kills actively replicating cells by inhibiting the depolymerization of microtubules. Why are microtubules good targets for killing cancerous cells?

11. During what phases in the cell cycle would you expect there to be large changes in the polymerization or depolymerization of microtubules? Why are these changes necessary?
12. When actively growing cells are treated with Taxol, they often are unable to complete the cell cycle. Based on what you have learned about cell-cycle checkpoints, which checkpoint likely causes these cells to arrest? Explain your reasoning.
13. **QUANTITATIVE** Suppose you performed the pulse–chase experiment illustrated in Figure 12.2 but included Taxol in the medium during the chase. Draw a new line on the graph to show the results you would expect, and explain why you would expect them.
14. **PROCESS OF SCIENCE** Aggressive forms of breast cancer are resistant to Taxol chemotherapy. In these cancers, the gene encoding a protein called stathmin is overexpressed. Scientists at Mount Sinai Hospital, New York, investigated the mechanism of action of stathmin. They measured tumour volume over time in mice with aggressive cancers under three conditions: no treatment (control), Taxol treatment, and Taxol treatment with stathmin gene expression turned off (Taxol + Δ stathmin). Their results are shown below. Use these results to hypothesize how the stathmin protein affects microtubule stability.



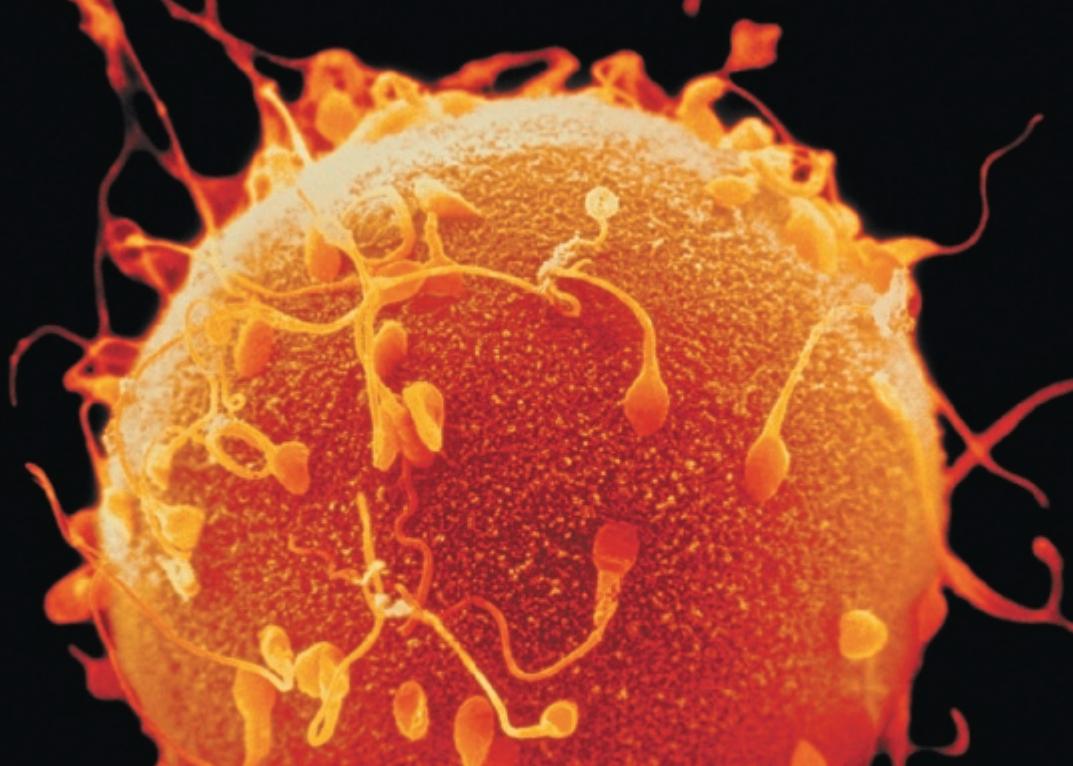
Source: Based on C. Miceli et al. 2013. *Cancer Gene Therapy* 20: 298–307.

15. In normal cells, stathmin is inactivated by phosphorylation at the start of M phase. Phosphatases remove these phosphates as the cell transitions from M phase to G₁. What enzyme is likely to be responsible for phosphorylating stathmin during M phase?
16. Inhibiting expression of the stathmin gene arrests cells in M phase and is being investigated as an alternative therapy for treating cancer. What additional genes could be therapeutic targets that, when inactivated, would arrest cancerous cells in G₁ phase?

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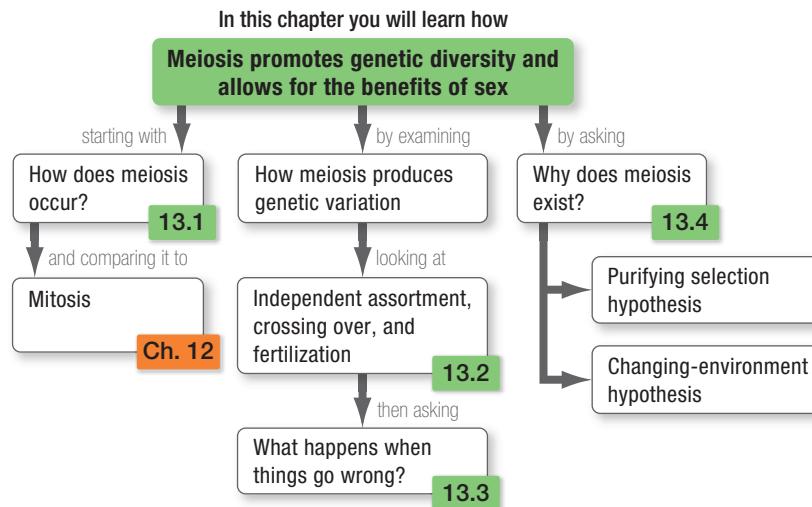
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David Phillips/Photo Researchers, Inc./Science Source

13 Meiosis

Scanning electron micrograph (with colour added) showing human sperm attempting to enter a human egg. This chapter introduces the type of nuclear division called meiosis, which in animals occurs before sperm and eggs are formed.



W hy sex?

Simple questions—such as why sexual reproduction exists—are sometimes the best for getting to the heart of things. This chapter asks what sexual reproduction is and why some organisms employ it. The focus here is on how organisms reproduce, or replicate—one of the five fundamental attributes of life introduced in Chapter 1.

For centuries people have known that during sexual reproduction in animals, a male reproductive cell—a **sperm**—and a female reproductive cell—an **egg**—unite in a process called **fertilization** to form a new individual. The first biologists to observe fertilization studied the large, translucent eggs of sea urchins. Thanks to the semitransparency of these eggs, researchers were able to see the nuclei of a sperm and an egg fuse.

BIG PICTURE

This chapter is part of the Big Picture. See how on pages 408–409.