10.1 Bacterial Cell Division

Learning Outcome

1. *Describe the process of binary fission.*

Bacteria divide as a way of reproducing themselves. Although bacteria exchange DNA, they do not have a sexual cycle like eukaryotes. Thus all growth in a bacterial population is due to division to produce new cells. The reproduction of bacteria is clonal—that is, each cell produced by cell division is an identical copy of the original cell.

Binary fission is a simple form of cell division

Cell division in both bacterial and eukaryotic cells produces two new cells with the same genetic information as the original. Despite the differences in these cell types, the essentials of the process are the same: duplication and segregation of genetic information into daughter cells, and division of cellular contents. We begin by looking at the simpler process, **binary fission,** which occurs in bacteria.

Most bacteria have a genome made up of a single, circular DNA molecule. In spite of its apparent simplicity, the DNA molecule of the bacterium *Escherichia coli* is actually on the order of 500 times longer than the cell itself! Thus, this "simple" structure is actually packaged very tightly to fit into the cell. Although not found in a nucleus, the DNA is located in a region called the *nucleoid* that is distinct from the cytoplasm around it.

The compaction and organization of the nucleoid involves a class of proteins called structural maintenance of chromosome, or SMC, proteins. These are ancient proteins that have evolved to perform a number of roles related to DNA compression and cohesion. In eukaryotes the cohesin and condensin proteins discussed later in the chapter are SMC proteins.

During binary fission, the chromosome is replicated, and the two products are partitioned to each end of the cell prior to the actual division of the cell. One key feature of bacterial cell division is that replication and partitioning of the chromosome occur as a concerted process. In contrast, DNA replication in eukaryotic cells occurs early in division, and chromosome separation occurs much later.

Proteins control chromosome separation and septum formation

Binary fission begins with the replication of the bacterial DNA at a specific site—the origin of replication (see chapter 14)—and proceeds both directions around the circular DNA to a specific site of termination (figure 10.1). The cell grows by elongation, and division occurs roughly at midcell. For many years, it was thought that newly replicated *E. coli* DNA molecules were passively segregated by attachment to and growth of the membrane as the cell elongated. Experiments that follow the movement of the origin of replication show that it is at

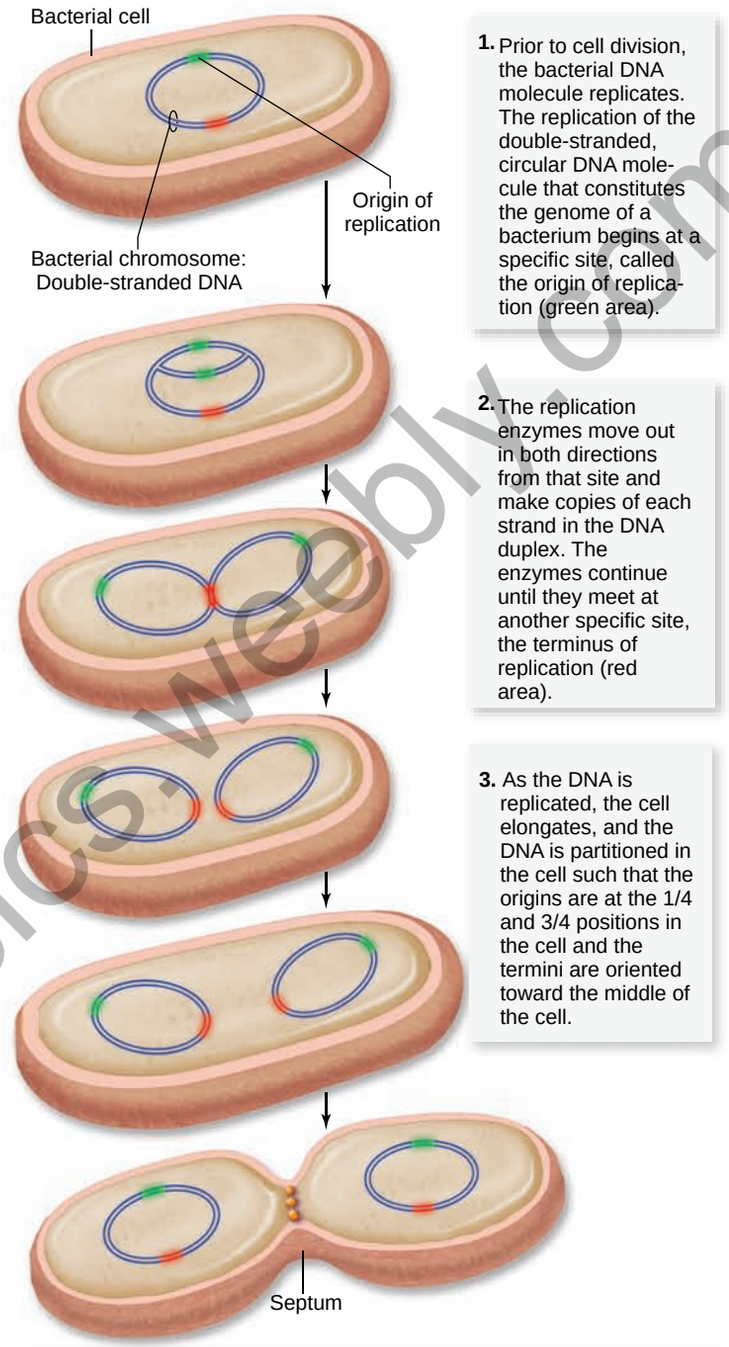


Figure 10.1 Binary fission.

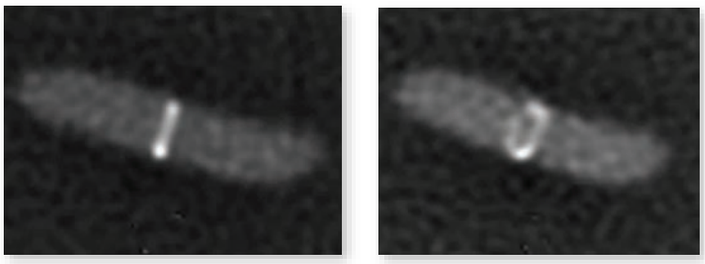


Figure 10.2 The FtsZ protein. In these dividing *E. coli* bacteria, the FtsZ protein is labeled with fluorescent dye to show its location during binary fission. The protein assembles into a ring at approximately the midpoint of the cell, where it facilitates septation and cell division. Bacteria carrying mutations in the *FtsZ* gene are unable to divide.

midcell prior to replication, then the newly replicated origins move toward opposite ends of the cell. This movement is faster than the rate of elongation, showing that growth alone is not enough. The origins appear to be captured at the one quarter and three quarter positions relative to the length of the cell, which will be midcell of the two daughter cells.

Although the actual mechanism of chromosome segregation is unclear, the order of events is not. During replication, first the origin, then the rest of the newly replicated chromosomes are moved to opposite ends of the cell as two new nucleoids are assembled. The final event of replication is decatenation (untangling) of the final replication products. After replication and segregation, the midcell region is cleared of daughter nucleoids, and division occurs. The force behind chromosome segregation has been attributed to DNA replication itself, transcription, and the polymerization of actin-like molecules. At this point, no single model appears to explain the process, and it may involve more than one.

The cell's other components are partitioned by the growth of new membrane and production of the **septum** (see figure 10.1). This process, termed **septation,** usually occurs at the midpoint of the cell. It begins with the formation of a ring composed of many copies of the protein FtsZ (figure 10.2). Next, accumulation of a number of other proteins occurs, including ones embedded in the membrane. This structure contracts inward radially until the cell pinches off into two new cells. The midcell location of the FtsZ ring is caused by an oscillation between the two poles of an inhibitor of FtsZ formation.

The FtsZ protein is found in most prokaryotes, including archaea. It can form filaments and rings, and recent three-dimensional crystals show a high degree of similarity to eukaryotic tubulin. However, its role in bacterial division is quite different from the role of tubulin in mitosis in eukaryotes.

The evolution of eukaryotic cells included much more complex genomes composed of multiple linear chromosomes housed in a membrane-bounded nucleus. These complex genomes may be possible due to the evolution of mechanisms that delay chromosome separation after replication. Although it is unclear how this ability to keep chromosomes together evolved, it does seem more closely related to binary fission than we once thought (figure 10.3).

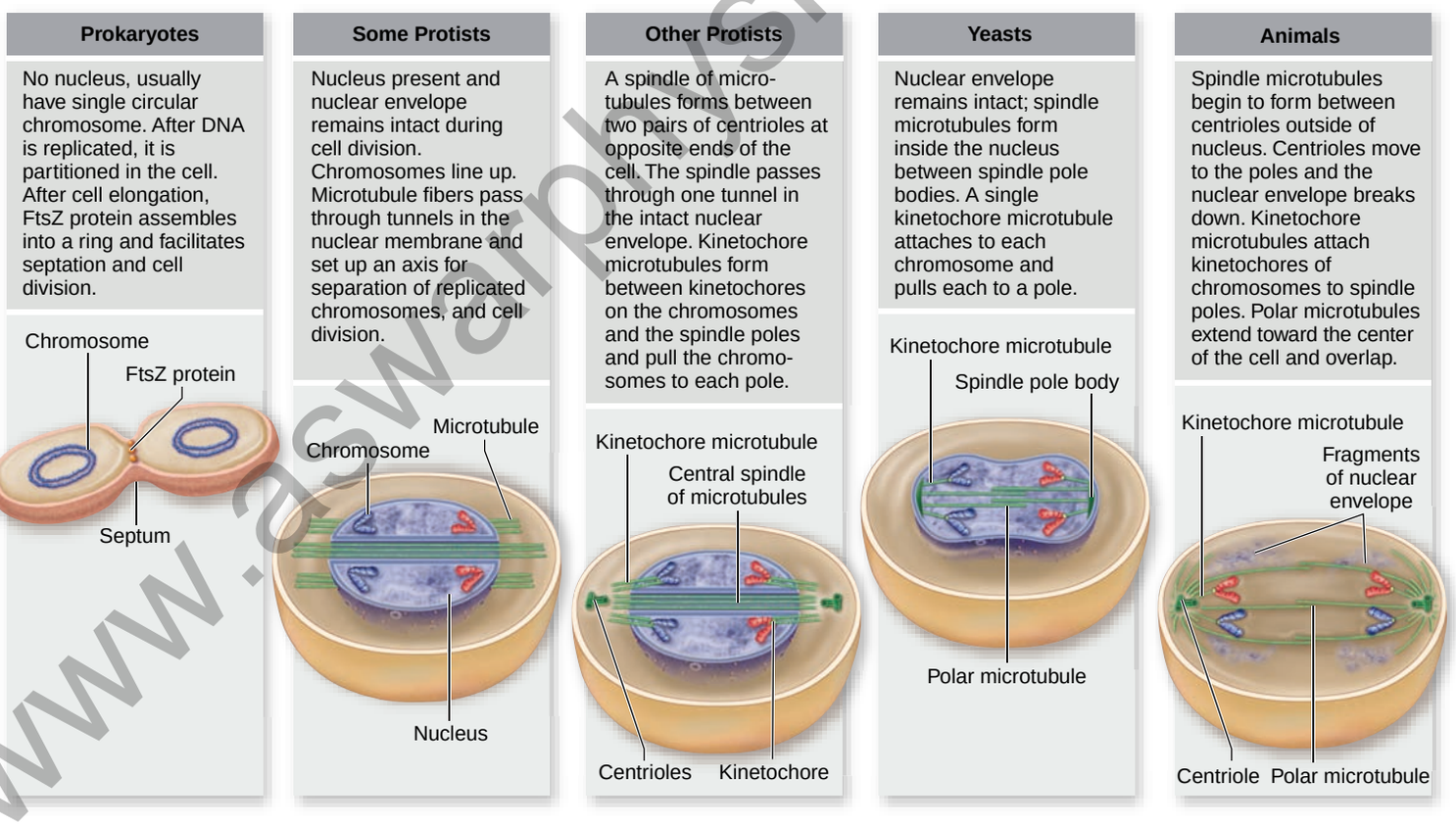


Figure 10.3 A comparison of protein assemblies during cell division among different organisms. The prokaryotic protein FtsZ has a structure that is similar to that of the eukaryotic protein tubulin. Tubulin is the protein component of microtubules, which are fibers used to separate chromosomes in eukaryotic cell division.

Learning Outcome Review 10.1

Most bacteria divide by binary fission, a form of cell division in which DNA replication and segregation occur simultaneously. This process involves active partitioning of the single bacterial chromosome and positioning of the site of septation.

- ***Would binary fission work as well if bacteria had many chromosomes?***

10.2 Eukaryotic Chromosomes

Learning Outcomes

1. ***Describe the structure of eukaryotic chromosomes.***
2. ***Distinguish between homologues and sister chromatids.***

TABLE 10.1 Chromosome Number in Selected Eukaryotes

Group	Total Number of Chromosomes
FUNGI	
Neurospora (haploid)	7
Saccharomyces (a yeast)	16
INSECTS	
Mosquito	6
Drosophila	8
Honeybee	diploid females 32, haploid males 16
Silkworm	56
PLANTS	
Haplopappus gracilis	2
Garden pea	14
Corn	20
Bread wheat	42
Sugarcane	80
Horsetail	216
Adder's tongue fern	1262
VERTEBRATES	
Opossum	22
Frog	26
Mouse	40
Human	46
Chimpanzee	48
Horse	64
Chicken	78
Dog	78

Chromosomes were first observed by the German embryologist Walther Flemming (1843–1905) in 1879, while he was examining the rapidly dividing cells of salamander larvae. When Flemming looked at the cells through what would now be a rather primitive light microscope, he saw minute threads within their nuclei that appeared to be dividing lengthwise. Flemming called their division **mitosis,** based on the Greek word *mitos*, meaning "thread."

Chromosome number varies among species

Since their initial discovery, chromosomes have been found in the cells of all eukaryotes examined. Their number may vary enormously from one species to another. A few kinds of organisms have only a single pair of chromosomes, whereas some ferns have more than 500 pairs (table 10.1). Most eukaryotes have between 10 and 50 chromosomes in their body cells.

Human cells each have 46 chromosomes, consisting of 23 nearly identical pairs (figure 10.4). Each of these 46 chromosomes contains hundreds or thousands of genes that play important roles in determining how a person's body develops and functions. Human embryos missing even one chromosome, a condition called *monosomy*, do not survive in most cases. Having an extra copy of any one chromosome, a condition called *trisomy*, is usually fatal except where the smallest chromosomes are involved. (You'll learn more about human chromosome abnormalities in chapter 13.)

Eukaryotic chromosomes exhibit complex structure

Researchers have learned a great deal about chromosome structure and composition in the more than 125 years since their

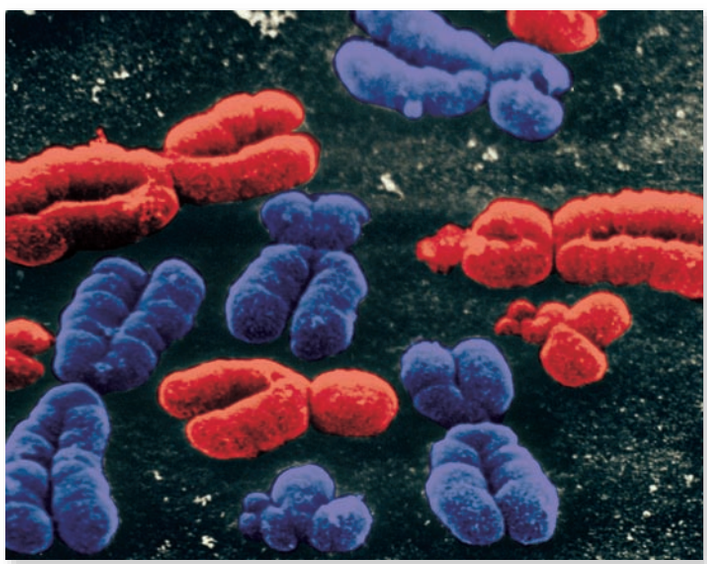


Figure 10.4 Human chromosomes. This scanning electron micrograph shows human chromosomes as they appear immediately before nuclear division. Each DNA molecule has already replicated, forming identical copies held together at a visible constriction called the centromere. False color has been added to the chromosomes.

discovery. But despite intense research, the exact structure of eukaryotic chromosomes during the cell cycle remains unclear. The structures described in this chapter represent the currently accepted model.

Composition of chromatin

Chromosomes are composed of *chromatin*, a complex of DNA and protein; most chromosomes are about 40% DNA and 60% protein. A significant amount of RNA is also associated with chromosomes because chromosomes are the sites of RNA synthesis.

The DNA of a single chromosome is one very long, double-stranded fiber that extends unbroken through the chromosome's entire length. A typical human chromosome contains about 140 million (1.4×10^8) nucleotides in its DNA. If we think of each nucleotide as a "word," then the amount of information an average chromosome contains would fill about 280 printed books of 1000 pages each, with 500 "words" per page.

If we could lay out the strand of DNA from a single chromosome in a straight line, it would be about 5 cm (2 in.) long. Fitting such a strand into a cell nucleus is like cramming a string the length of a football field into a baseball—and that's only 1 of 46 chromosomes! In the cell, however, the DNA is coiled, allowing it to fit into a much smaller space than would otherwise be possible.

The organization of chromatin in the nondividing nucleus is not well understood, but geneticists have recognized for years that some domains of chromatin, called **heterochromatin,** are not expressed, and other domains of chromatin, called **euchromatin,** are expressed. This genetically measurable state is also related to the physical state of chromatin, although researchers are just beginning to see the details.

Chromosome structure

If we gently disrupt a eukaryotic nucleus and examine the DNA with an electron microscope, we find that it resembles a string of beads (figure 10.5). Every 200 nucleotides (nt), the DNA duplex (double strand) is coiled around a core of eight **histone proteins.** Unlike most proteins, which have an overall negative charge, histones are positively charged because of an abundance of the basic amino acids arginine and lysine. Thus, they are strongly attracted to the negatively charged phosphate groups of the DNA, and the histone cores act as "magnetic forms" that promote and guide the coiling of the DNA. The complex of DNA and histone proteins is termed a **nucleosome.**

Further coiling occurs when the string of nucleosomes is wrapped into higher order coils called *solenoids.* The precise path of this higher order folding of chromatin is still a subject of some debate, but it leads to a fiber with a diameter of 30 nm and thus is often called the 30-nm fiber. This 30-nm fiber is the usual state of interphase (nondividing) chromatin.

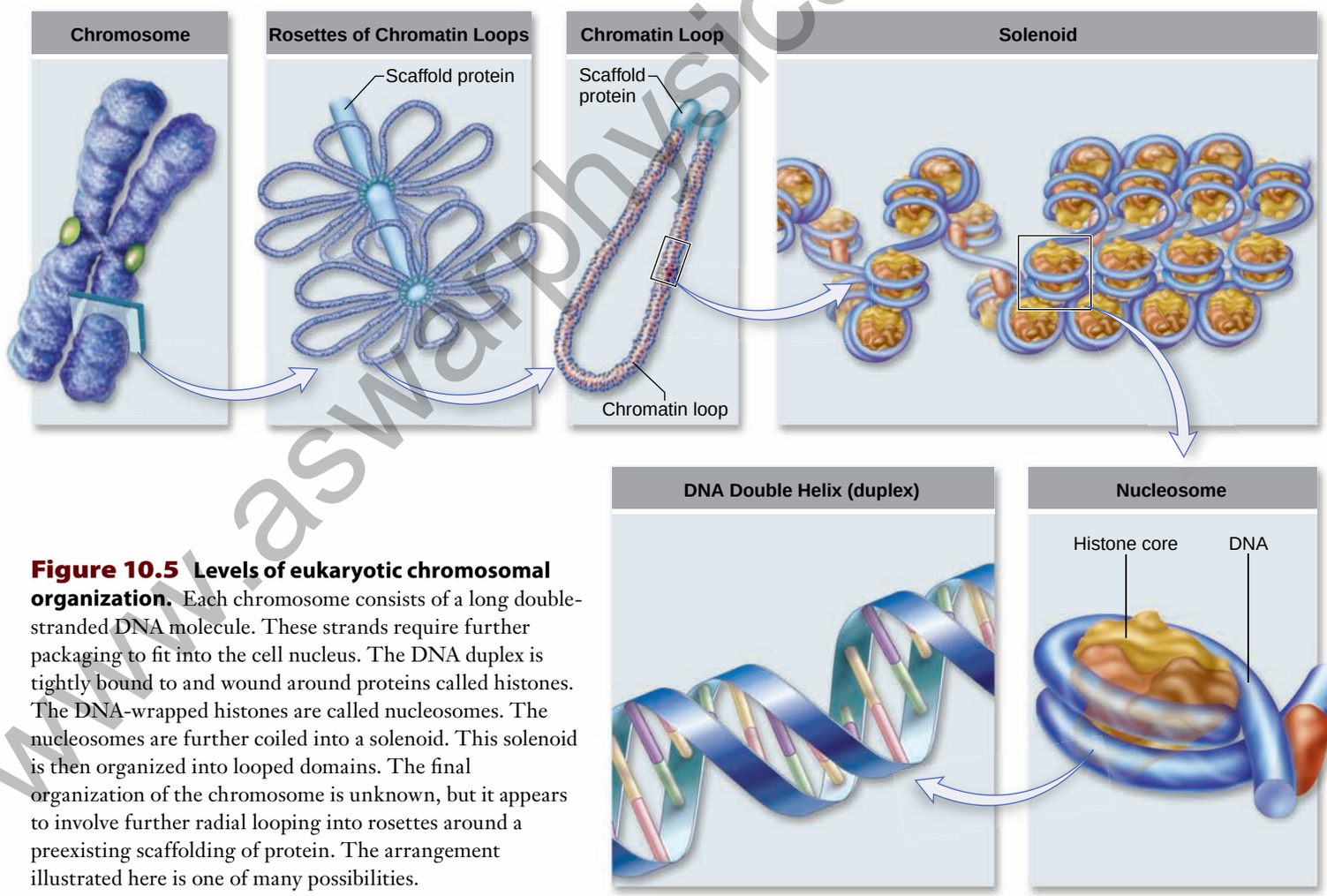


Figure 10.5 Levels of eukaryotic chromosomal organization. Each chromosome consists of a long double-stranded DNA molecule. These strands require further packaging to fit into the cell nucleus. The DNA duplex is tightly bound to and wound around proteins called histones. The DNA-wrapped histones are called nucleosomes. The nucleosomes are further coiled into a solenoid. This solenoid is then organized into looped domains. The final organization of the chromosome is unknown, but it appears to involve further radial looping into rosettes around a preexisting scaffolding of protein. The arrangement illustrated here is one of many possibilities.

During mitosis the chromatin in the solenoid is arranged around a scaffold of protein assembled at this time to achieve maximum compaction of the chromosomes. This process prepares the chromosomes for the events of mitosis described later. The exact nature of this compaction is unknown, but one long-standing model involves radial looping of the solenoid about the protein scaffold, aided by a complex of proteins called **condensin.**

Chromosome karyotypes

Chromosomes vary in size, staining properties, the location of the centromere (a constriction found on all chromosomes, described shortly), the relative length of the two arms on either side of the centromere, and the positions of constricted regions along the arms. The particular array of chromosomes an individual organism possesses is called its **karyotype.** The karyotype in figure 10.6 shows the set of chromosomes from a normal human cell.

When defining the number of different chromosomes in a species, geneticists count the **haploid *(n)*** number of chromosomes. This refers to one complete set of chromosomes necessary to define an organism. For humans and many other species, the total number of chromosomes in a cell is called the **diploid *(2n)*** number, which is twice the haploid number. For humans, the haploid number is 23 and the diploid number is 46. Diploid chromosomes reflect the equal genetic contribution that each parent makes to offspring. We refer to the maternal and paternal chromosomes as being **homologous,** and each one of the pair is termed a **homologue.**

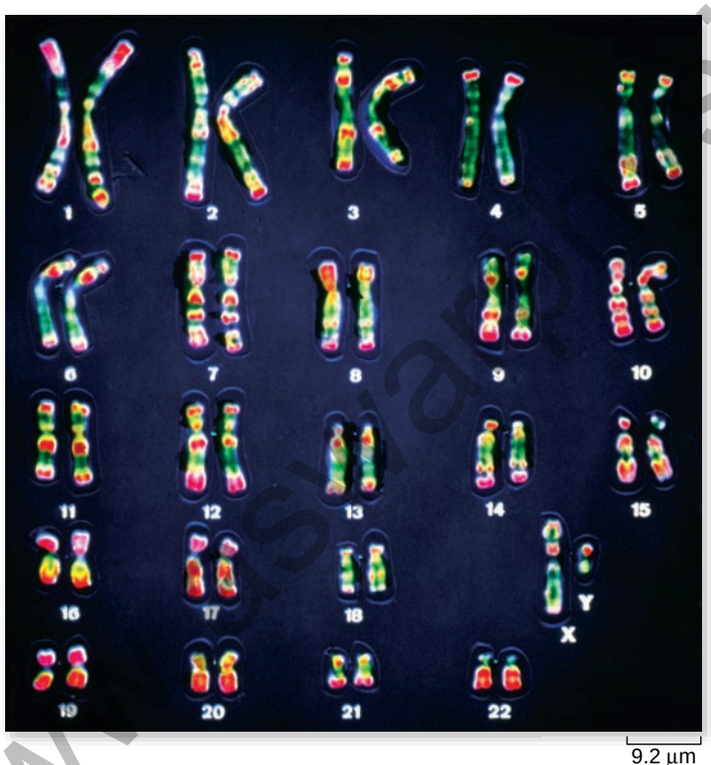


Figure 10.6 A human karyotype. The individual chromosomes that make up the 23 pairs differ widely in size and in centromere position. In this preparation of a male karyotype, the chromosomes have been specifically stained to indicate differences in their composition and to distinguish them clearly from one another. Notice that members of a chromosome pair are very similar but not identical.

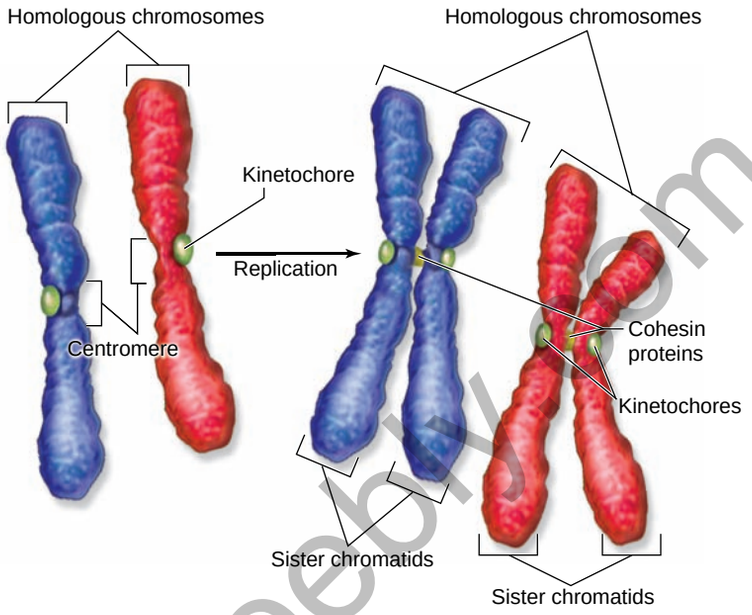


Figure 10.7 The difference between homologous chromosomes and sister chromatids. Homologous chromosomes are the maternal and paternal copies of the same chromosome—say, chromosome number 16. Sister chromatids are the two replicas of a single chromosome held together at their centromeres by cohesin proteins after DNA replication. The kinetochore (described later in the chapter) is composed of proteins found at the centromere that attach to microtubules during mitosis.

Chromosome replication

Chromosomes as seen in a karyotype are only present for a brief period during cell division. Prior to replicating, each chromosome is composed of a single DNA molecule that is arranged into the 30-nm fiber described earlier. After replication, each chromosome is composed of two identical DNA molecules held together by a complex of proteins called **cohesins.** As the chromosomes become more condensed and arranged about the protein scaffold, they become visible as two strands that are held together. At this point, we still call this one chromosome, but it is composed of two sister **chromatids** (figure 10.7).

The fact that the products of replication are held together is critical to the division process. One problem that a cell must solve is how to ensure that each new cell receives a complete set of chromosomes. If we were designing a system, we might use some kind of label to identify each chromosome, much like most of us use when we duplicate files on a computer. The cell has no mechanism to label chromosomes; instead, it keeps the products of replication together until the moment of chromosome segregation, ensuring that one copy of each chromosome goes to each daughter cell. This separation of sister chromatids is the key event in the mitotic process described in detail shortly.

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 using the metaphor of a clock face (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, mitochondria and other organelles replicate, and microtubules begin to assemble at 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

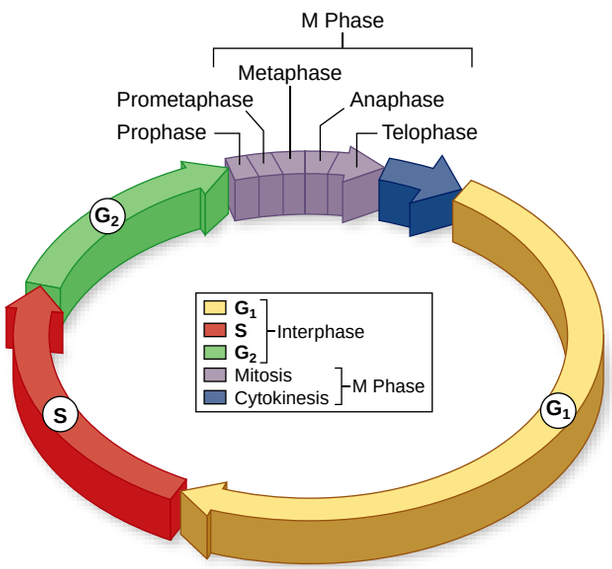


Figure 10.8 The cell cycle. The cell cycle is depicted as a circle. The first gap phase, G_1, involves growth and preparation for DNA synthesis. During S phase, a copy of the genome is synthesized. The second gap phase, G_2, prepares the cell for mitosis. During mitosis, replicated chromosomes are partitioned.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

days to years before resuming cell division. At any given time, most of the cells in an 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. ***Explain the structure of the centromere 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 certain 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. Functionally, however, the two chromatids have a single centromere due to their being attached by cohesin proteins at the centromere site (figure 10.10). In metazoan animals, most of the cohesins that hold sister chromatids together after replication appear to be replaced by condensin during the process of chromosome compaction. 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.

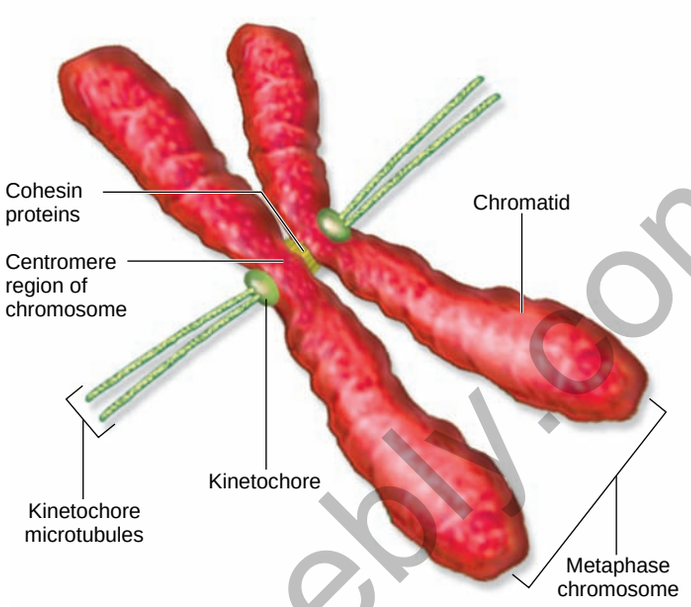


Figure 10.9 Kinetochores. Separation of sister chromatids during mitosis depends on microtubules attaching to proteins found in the kinetochore. These kinetochore proteins are assembled on the centromere of chromosomes. The centromeres of the two sister chromatids are held together by cohesin proteins.

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

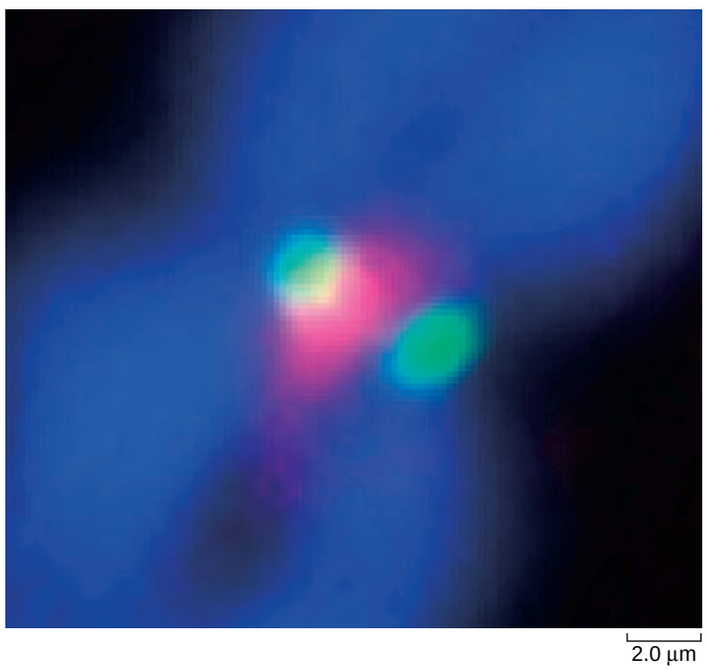


Figure 10.10 Proteins found at the centromere. In this image DNA, a cohesin protein and a kinetochore protein have all been labeled with a different colored fluorescent dye. Cohesin *(red)*, which holds centromeres together, lies between the sister chromatids *(blue)*. Each sister chromatid has its own separate kinetochore *(green)*.

the 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. *Understand the importance of chromatid cohesion.*
3. *Compare cytokinesis in plants and animals.*

The process of mitosis is one of the most dramatic and beautiful biological processes that can be readily observed. 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.

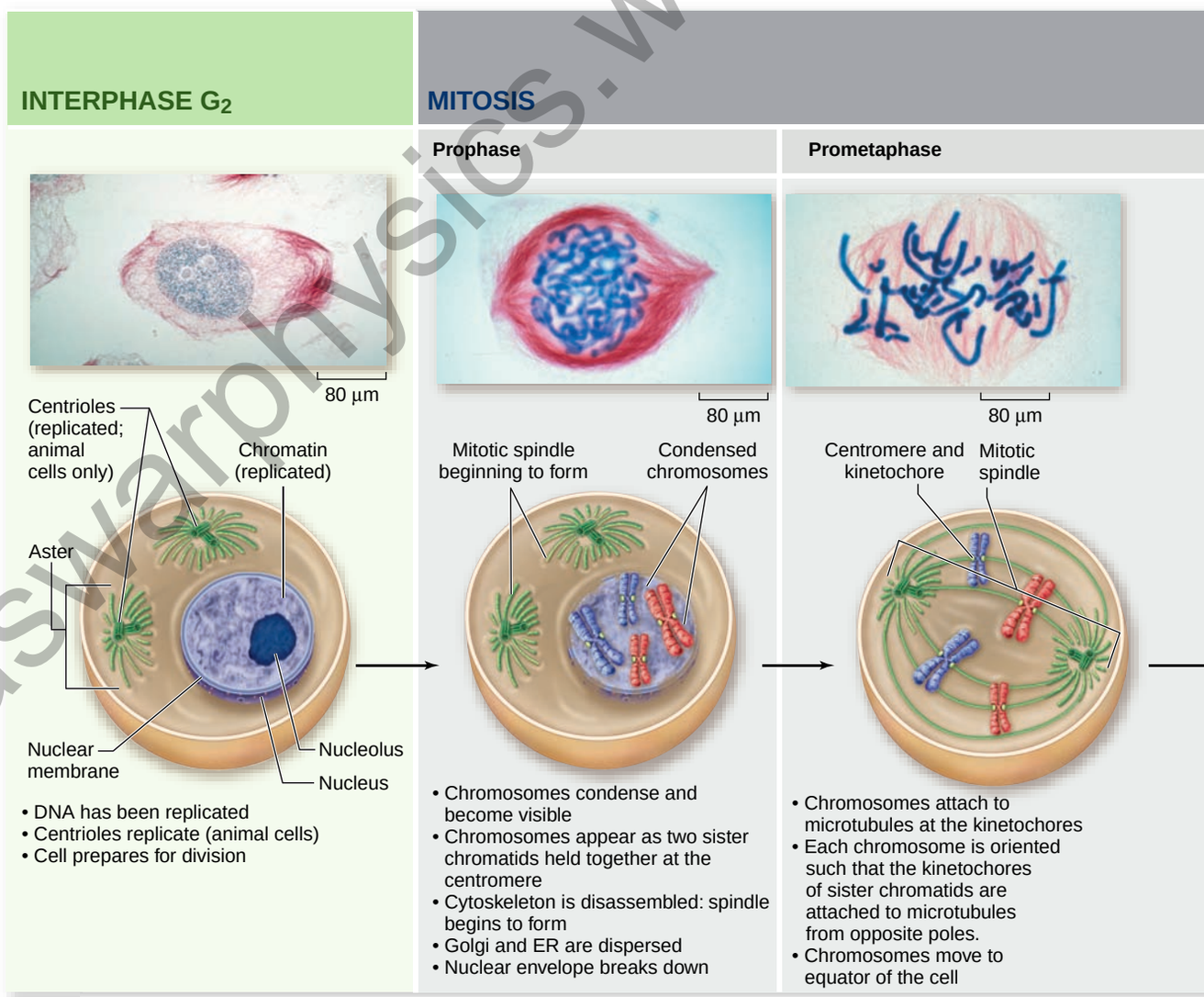


Figure 10.11 Mitosis and cytokinesis. Mitosis is conventionally divided into five stages—prophase, prometaphase, metaphase, anaphase, and telophase—which together act to separate duplicated chromosomes. This is followed by cytokinesis, which divides the cell into two separate cells. Photos depict mitosis 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.

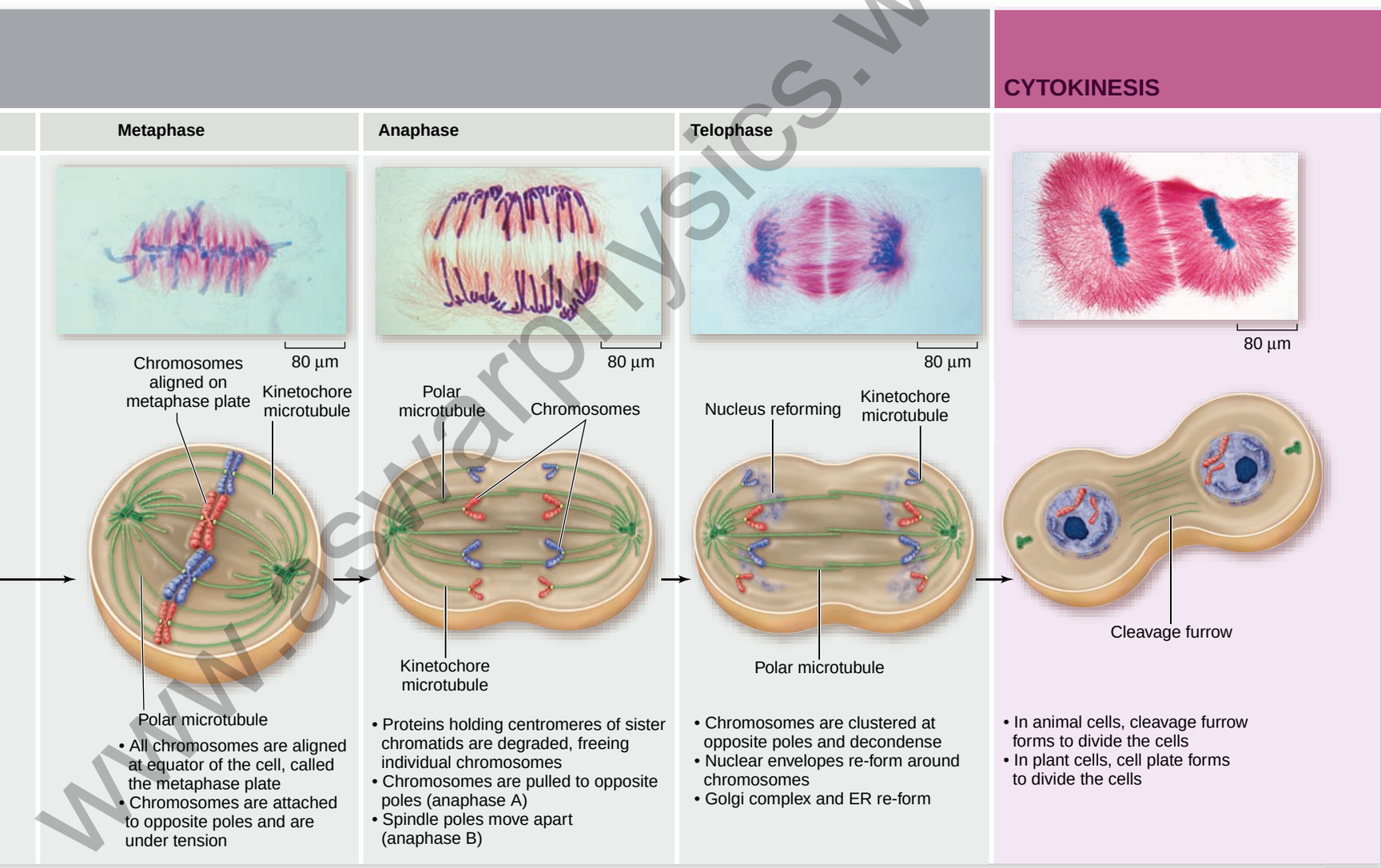
The spindle and centrioles

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

With each chromosome attached to the spindle by microtubules from opposite poles to the kinetochores of sister chromatids, the chromosomes begin to move to the center of the cell. This movement is jerky, as if a chromosome is being pulled toward both poles at the same time. This process is called *congression*, and it eventually leads to all of the chromosomes being arranged at the equator of the cell with the sister chromatids of each chromosome oriented to 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.

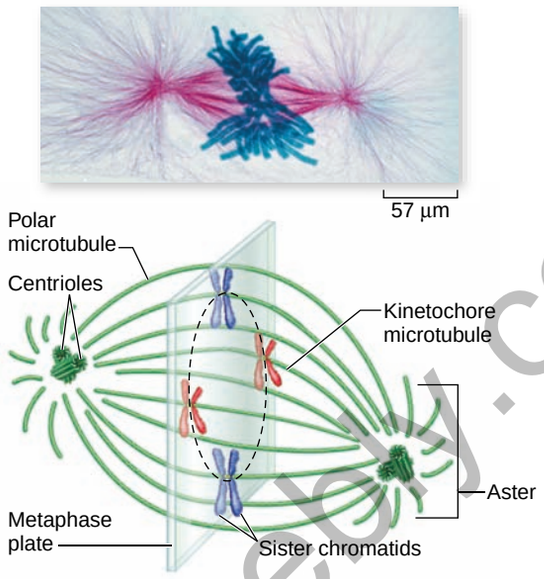


Figure 10.12 Metaphase. In metaphase, the chromosomes are arrayed at the midpoint of the cell. The imaginary plane through the equator of the cell is called the metaphase plate. As the spindle itself is a three dimensional structure, the chromosomes are arrayed in a rough circle on the metaphase plate.

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 centromeres split, freeing the two sister chromatids from each other. Up to this point in mitosis, sister chromatids have been held together by cohesin proteins concentrated at the centromere, as mentioned earlier. 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 later on 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.

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.

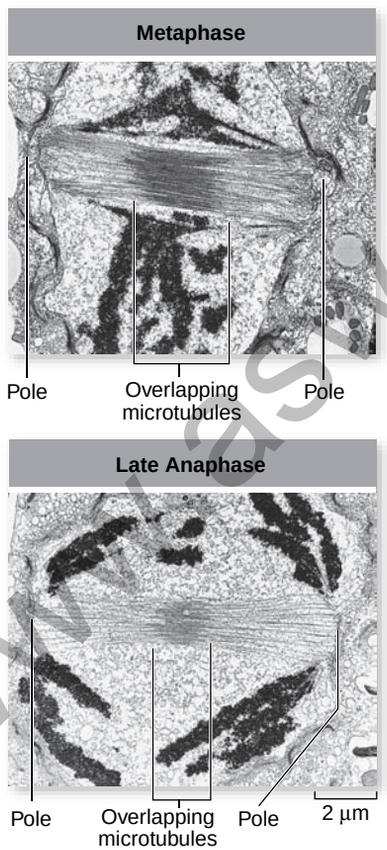


Figure 10.13 Microtubules slide past each other as the chromosomes separate. In these electron micrographs of dividing diatoms, the overlap of the microtubules lessens markedly during spindle elongation as the cell passes from metaphase to anaphase. During anaphase B the poles move farther apart as the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

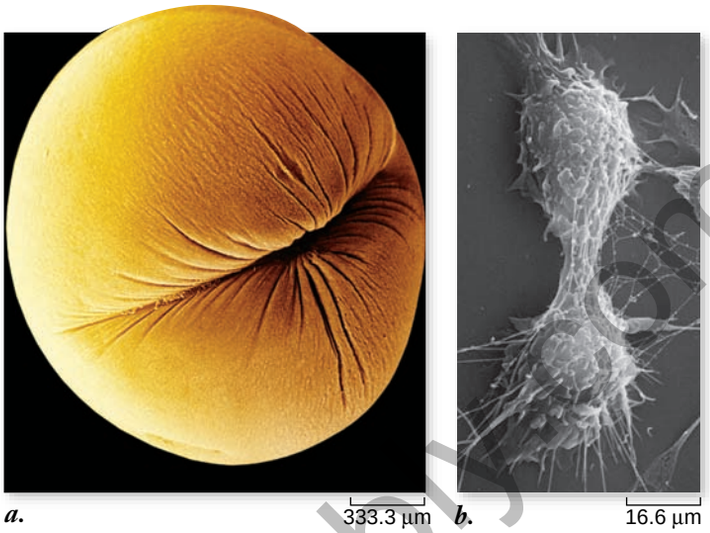


Figure 10.14 Cytokinesis in animal cells. ***a.*** A cleavage furrow forms around a dividing frog egg. ***b.*** The completion of cytokinesis in an animal cell. The two daughter cells are still joined by a thin band of cytoplasm occupied largely by microtubules.

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

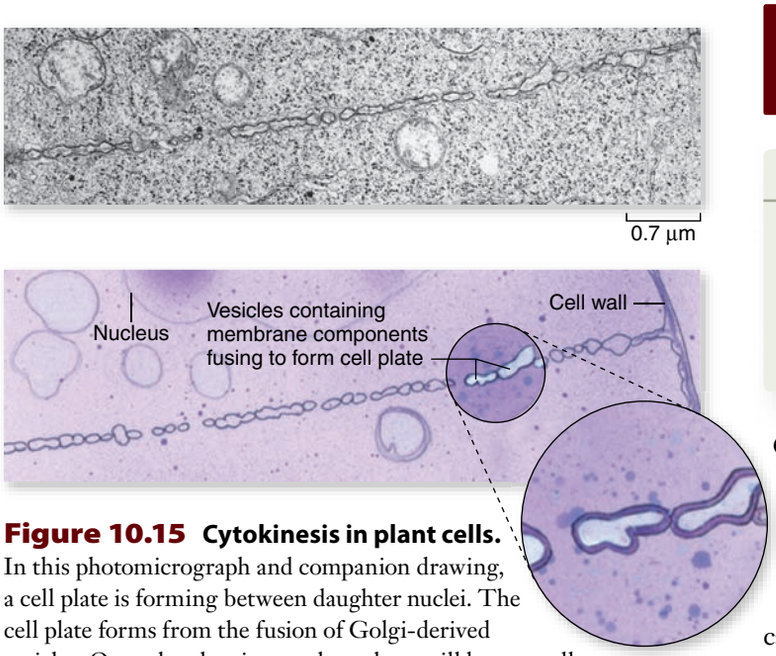


Figure 10.15 Cytokinesis in plant cells. In this photomicrograph and companion drawing, a cell plate is forming between daughter nuclei. The cell plate forms from the fusion of Golgi-derived vesicles. Once the plate is complete, there will be two cells.

(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 organisms does the nucleus divide into two daughter nuclei; then, during cytokinesis, one nucleus goes to each daughter cell. This separate nuclear division phase of the cell cycle does not occur in plants, animals, or most protists.

After cytokinesis in any eukaryotic cell, the two daughter cells contain all the components of a complete cell. Whereas mitosis ensures that both daughter cells contain a full complement of chromosomes, no similar mechanism ensures that organelles such as mitochondria and chloroplasts are distributed equally between the daughter cells. But as long as at least one of each organelle is present in each cell, the organelles can replicate to reach the number appropriate for that cell.

Learning Outcomes Review 10.5

Mitosis is divided into phases: prophase, prometaphase, metaphase, anaphase, and telophase. The early phases involve restructuring the cell to create the microtubule spindle that pulls chromosomes to the equator of the cell in metaphase. Chromatids for each chromosome remain attached at the centromere by cohesin proteins. Chromatids are then pulled to opposite poles during anaphase when cohesin proteins are destroyed. The nucleus is re-formed in telophase, and cytokinesis then divides the cell cytoplasm and organelles. In animal cells, actin pinches the cell in two; in plant cells, a cell plate forms in the middle of the dividing cell.

- ***What would happen to a chromosome that loses cohesin protein between sister chromatids before metaphase?***

10.6 Control of the Cell Cycle

Learning Outcomes

1. ***Distinguish the role of checkpoints in the control of the cell cycle.***
2. ***Understand the role of the anaphase-promoting complex/cyclosome in mitosis.***
3. ***Describe cancer in terms of cell cycle control.***

Our knowledge of how the cell cycle is controlled, although still incomplete, has grown enormously in the past 30 years. Our current view integrates two basic concepts. First, the cell cycle has two irreversible points: the replication of genetic material and the separation of the sister chromatids. Second, the cell cycle can be put on hold at specific points called *checkpoints*. At any of these checkpoints, the process is checked for accuracy and can be halted if there are errors. This leads to extremely high fidelity overall for the entire process. The checkpoint organization also allows the cell cycle to respond to both the internal state of the cell, including nutritional state and integrity of genetic material, and to signals from the environment, which are integrated at major checkpoints.

Research uncovered cell cycle control factors

The history of investigation into control of the cell cycle is instructive in two ways. First, it allows us to place modern observations into context; second, we can see how biologists using very different approaches often end up at the same place. The following brief history introduces three observations and then shows how they can be integrated into a single mechanism.

Discovery of MPF

Research on the activation of frog oocytes led to the discovery of a substance that was first called *maturation-promoting factor (MPF)*. Frog oocytes, which go on to become egg cells, become arrested near the end of their development at the G_2 stage before meiosis I, which is the division leading to the production of gametes (chapter 11). They remain in this arrested state and await hormonal signaling to complete this division process.

Cytoplasm taken from a variety of actively dividing cells could prematurely induce cell division when injected into oocytes (figure 10.16). These experiments indicated the presence of a positive regulator of cell cycle progression in the cytoplasm of dividing cells: MPF. These experiments also fit well with cell fusion experiments done with mitotic and interphase cells that also indicated a cytoplasmic positive regulator that could induce mitosis (figure 10.16).

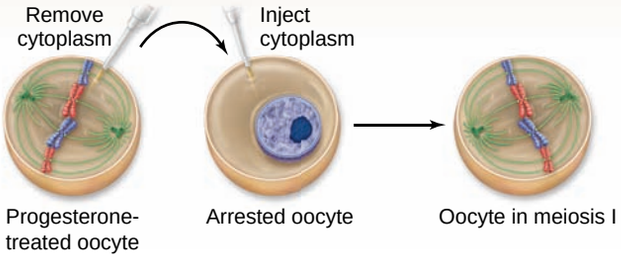
Further studies highlighted two key aspects of MPF. First, MPF activity varied during the cell cycle: low in early G_2, rising throughout this phase, and then peaking in mitosis (figure 10.17). Second, the enzymatic activity of MPF involved

SCIENTIFIC THINKING

Hypothesis: *There are positive regulators of cell division.*

Prediction: *Frog oocytes are arrested in G_2 of meiosis I. They can be induced to mature (undergo meiosis) by progesterone treatment. If maturing oocytes contain a positive regulator of cell division, injection of cytoplasm should induce an immature oocyte to undergo meiosis.*

Test: *Oocytes are induced with progesterone, then cytoplasm from these maturing cells is injected into immature oocytes.*

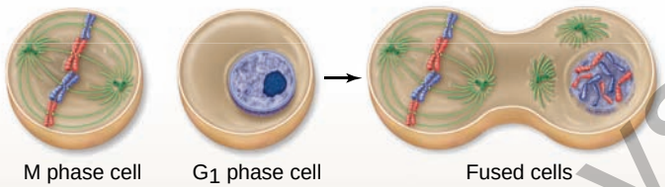


Result: *Injected oocytes progress from G_2 into meiosis I.*

Conclusion: *The progesterone treatment causes production of a positive regulator of maturation: Maturation Promoting Factor (MPF).*

Prediction: *If mitosis is driven by positive regulators, then cytoplasm from a mitotic cell should cause a G_1 cell to enter mitosis.*

Test: *M phase cells are fused with G_1 phase cells, then the nucleus from the G_1 phase cell is monitored microscopically.*



Conclusion: *Cytoplasm from M phase cells contains a positive regulator that causes a cell to enter mitosis.*

Further Experiments: *How can both of these experiments be rationalized? What would be the next step in characterizing these factors?*

Figure 10.16 Discovery of positive regulator of cell division.

the phosphorylation of proteins. This second point is not surprising given the importance of phosphorylation as a reversible switch on the activity of proteins (see chapter 9). The first observation indicated that MPF itself was not always active, but rather was being regulated with the cell cycle, and the second showed the possible enzymatic activity of MPF.

Discovery of cyclins

Other researchers examined proteins produced during the early divisions in sea urchin embryos. They identified proteins that were produced in synchrony with the cell cycle, and named them **cyclins** (see figure 10.17). These observations were extended in another marine invertebrate, the surf clam. Two forms of cyclin were found that cycled at slightly different times, reaching peaks at the G_1/S and G_2/M boundaries.

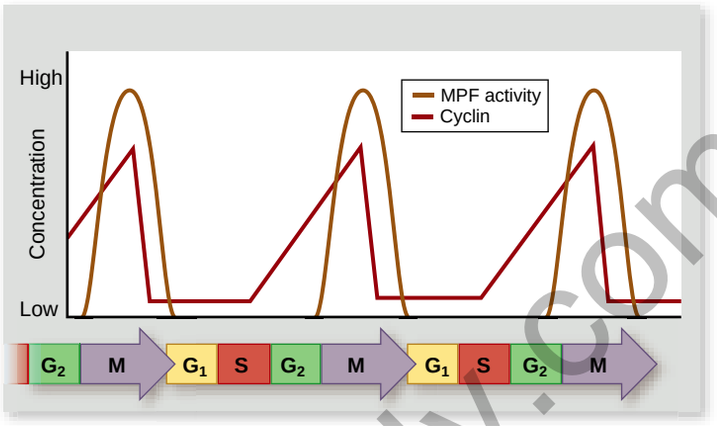


Figure 10.17 Correlation of MPF activity, amount of cyclin protein, and stages of the cell cycle. Cyclin concentration and MPF activity are shown plotted vs. stage of the cell cycle. MPF activity changes in a repeating pattern through the cell cycle. This also correlates with the level of mitotic cyclin in the cell, which shows a similar pattern. The reason for this correlation is that cyclin is actually one component of MPF, the other being a cyclin-dependent kinase (Cdk). Together these act as a positive regulator of cell division.

Despite much effort, no identified enzymatic activity was associated with these proteins. Their hallmark was the timing of their production and not any intrinsic activity.

Genetic analysis of the cell cycle

Geneticists using two different yeasts, budding yeast and fission yeast, as model systems set out to determine the genes necessary for control of the cell cycle. By isolating mutants that were halted during division, they identified genes that were necessary for cell cycle progression. These studies indicated that in yeast, there were two critical control points: the commitment to DNA synthesis, called START, as it meant committing to divide, and the commitment to mitosis. One particular gene, named *cdc2*, from fission yeast, was shown to be critical for passing both of these boundaries.

MPF is cyclin plus cdc2

All of these findings came together in an elegant fashion with the following observations. First, the protein encoded by the *cdc2* gene was shown to be a protein kinase. Second, the purification and identification of MPF showed that it was composed of both a cyclin component and a kinase component. Last, the kinase itself was the cdc2 protein!

The cdc2 protein was the first identified **cyclin-dependent kinase (Cdk),** that is, a protein kinase enzyme that is only active when complexed with cyclin. This finding led to the renaming of MPF as *mitosis*-promoting factor, as its role was clearly more general than simply promoting the maturation of frog oocytes.

These Cdk enzymes are the key positive drivers of the cell division cycle. They are often called the engine that drives cell division. The control of the cell cycle in higher eukaryotes

is much more complex than the simple single-engine cycle of yeast, but the yeast model remains a useful framework for understanding more complex regulation. The discovery of Cdks and their role in the cell cycle is an excellent example of the progressive nature of science.

The cell cycle can be halted at three checkpoints

Although we have divided the cell cycle into phases and subdivided mitosis into stages, the cell recognizes three points at which the cycle can be delayed or halted. The cell uses these three checkpoints to both assess its internal state and integrate external signals (figure 10.18): G_1/S, G_2/M, and late metaphase (the spindle checkpoint). Passage through these checkpoints is controlled by the Cdk enzymes described earlier and also in the following section.

G_1/S checkpoint

The **G_1/S checkpoint** is the primary point at which the cell "decides" whether or not to divide. This checkpoint is therefore the primary point at which external signals can influence events of the cycle. It is the phase during which growth factors (discussed later on) affect the cycle and also the phase that links cell division to cell growth and nutrition.

In yeast systems, where the majority of the genetic analysis of the cell cycle has been performed, this checkpoint is called START. In animals, it is called the restriction point (R point). In all systems, once a cell has made this irreversible commitment to replicate its genome, it has committed to divide. Damage to DNA can halt the cycle at this point, as can starvation conditions or lack of growth factors.

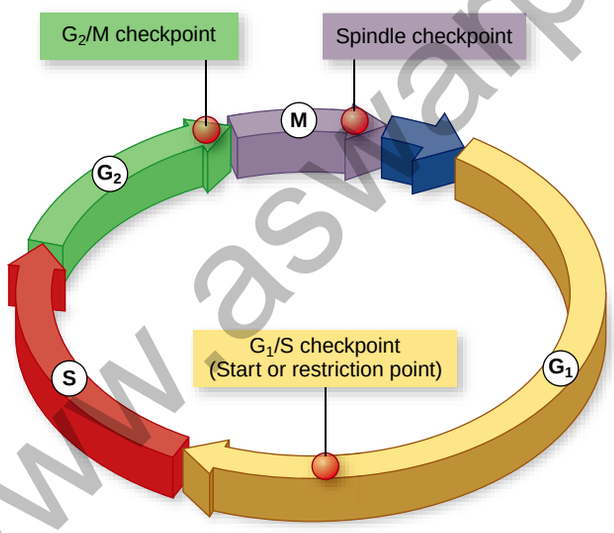


Figure 10.18 Control of the cell cycle. Cells use a centralized control system to check whether proper conditions have been achieved before passing three key checkpoints in the cell cycle.

G_2/M checkpoint

The **G_2/M checkpoint** has received a large amount of attention because of its complexity and its importance as the stimulus for the events of mitosis. Historically, Cdks active at this checkpoint were first identified as MPFs, a term that has now evolved into **M phase-promoting factor (MPF).**

Passage through this checkpoint represents the commitment to mitosis. This checkpoint assesses the success of DNA replication and can stall the cycle if DNA has not been accurately replicated. DNA-damaging agents result in arrest at this checkpoint as well as at the G_1/S checkpoint.

Spindle checkpoint

The **spindle checkpoint** ensures that all of the chromosomes are attached to the spindle in preparation for anaphase. The second irreversible step in the cycle is the separation of chromosomes during anaphase, and therefore it is critical that they are properly arrayed at the metaphase plate.

Cyclin-dependent kinases drive the cell cycle

The primary molecular mechanism of cell cycle control is phosphorylation, which you may recall is the addition of a phosphate group to the amino acids serine, threonine, and tyrosine in proteins (chapter 9). The enzymes that accomplish this phosphorylation are the Cdks (figure 10.19).

The action of Cdks

The first important cell cycle kinase was identified in fission yeast and named Cdc2 (now also called Cdk1). In yeast, this Cdk can partner with different cyclins at different points in the cell cycle (figure 10.20).

Even in the simplified cycle of the yeasts, we are left with the important question of what controls the activity of the Cdks during the cycle. For many years, a common view was that cyclins drove the cell cycle—that is, the periodic synthesis and destruction of cyclins acted as a clock. More recently, it has become clear that the Cdc2 kinase is also itself controlled by

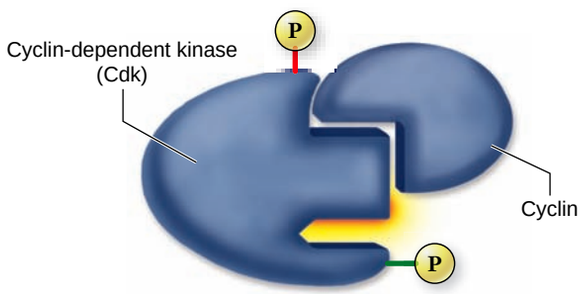


Figure 10.19 Cdk enzyme forms a complex with cyclin. Cdk is a protein kinase that activates numerous cell proteins by phosphorylating them. Cyclin is a regulatory protein required to activate Cdk. This complex is also called mitosis-promoting factor (MPF). The activity of Cdk is also controlled by the pattern of phosphorylation: phosphorylation at one site (represented by the red site) inactivates the Cdk, and phosphorylation at another site (represented by the green site) activates the Cdk.

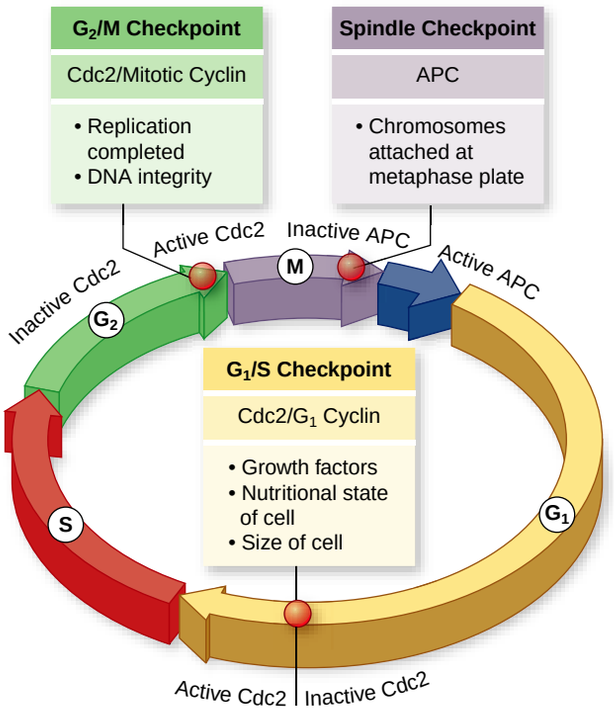


Figure 10.20 Checkpoints of the yeast cell cycle. The simplest cell cycle that has been studied in detail is the fission yeast. This is controlled by three main checkpoints and a single Cdk enzyme, called Cdc2. The Cdc2 enzyme partners with different cyclins to control the G_1/S and G_2/M checkpoints. The spindle checkpoint is controlled by the anaphase-promoting complex (APC).

phosphorylation: Phosphorylation at one site activates Cdc2, and phosphorylation at another site inactivates it (see figure 10.19). Full activation of the Cdc2 kinase requires complexing with a cyclin and the appropriate pattern of phosphorylation.

As the G_1/S checkpoint is approached, the triggering signal in yeast appears to be the accumulation of G_1 cyclins. These form a complex with Cdc2 to create the active G_1/S Cdk, which phosphorylates a number of targets that bring about the increased enzyme activity for DNA replication.

The action of MPF

MPF and its role at the G_2/M checkpoint has been extensively analyzed in a number of different experimental systems. The control of MPF is sensitive to agents that disrupt or delay replication and to agents that damage DNA. It was once thought that MPF was controlled solely by the level of the M phase-specific cyclins, but it has now become clear that this is not the case.

Although M phase cyclin is necessary for MPF function, activity is controlled by inhibitory phosphorylation of the kinase component, Cdc2. The critical signal in this process is the removal of the inhibitory phosphates by a protein, phosphatase. This action forms a molecular switch based on positive feedback because the active MPF further activates its own activating phosphatase.

The checkpoint assesses the balance of the kinase that adds inhibitory phosphates with the phosphatase that removes them. Damage to DNA acts through a complex pathway that includes damage sensing and a response to tip the balance toward the inhibitory phosphorylation of MPF. Later on, we describe how some cancers overcome this inhibition.

The anaphase-promoting complex

The molecular details of the sensing system at the spindle checkpoint are not clear. The presence of all chromosomes at the metaphase plate and the tension on the microtubules between opposite poles are both important. The signal is transmitted through the **anaphase-promoting complex,** also called the *cyclosome (APC/C)*.

The function of the APC/C is to trigger anaphase itself. As described earlier, the sister chromatids at metaphase are still held together by the protein complex cohesin. The APC does not act directly on cohesin, but rather acts by marking a protein called *securin* for destruction. The securin protein acts as an inhibitor of another protease called *separase* that appears to be specific for the cohesin complex. Once inhibition is lifted, separase destroys cohesin.

This process has been analyzed in detail in budding yeast, where it has been shown that the separase enzyme specifically degrades a component of cohesin called Scc1. This leads to the release of the sister chromatids and results in their sudden movement toward opposite poles during anaphase.

In vertebrates, most cohesin is removed from the sister chromatids during chromosome condensation, possibly with cohesin being replaced by condensin. At metaphase, the majority of the cohesin that remains on vertebrate chromatids is concentrated at the centromere (figure 10.10). The destruction of this cohesin explains the anaphase movement of chromosomes and the apparent "division" of the centromeres.

The APC/C has a number of roles in mitosis: it activates the protease that removes the cohesins holding sister chromatids together, and it is necessary for the destruction of mitotic cyclins to drive the cell out of mitosis. The APC/C complex marks proteins for destruction by the proteosome, the organelle responsible for the controlled degradation of proteins (chapter 16). The signal to degrade a protein is the addition of a molecule called *ubiquitin*, and the APC/C acts as a ubiquitin ligase. As we learn more about the APC/C and its functions, it is clear that the control of its activity is a key regulator of the cell cycle.

In multicellular eukaryotes, many Cdks and external signals act on the cell cycle

The major difference between more complex animals and single-celled eukaryotes such as fungi and protists is twofold: First, multiple Cdks control the cycle as opposed to the single Cdk in yeasts; and second, animal cells respond to a greater variety of external signals than do yeasts, which primarily respond to signals necessary for mating.

In higher eukaryotes there are more Cdk enzymes and more cyclins that can partner with these multiple Cdks, but their basic role is the same as in the yeast cycle. A more complex

cell cycle is shown in figure 10.21. These more complex controls allow the integration of more input into control of the cycle. With the evolution of more complex forms of organization (tissues, organs, and organ systems), more complex forms of cell cycle control evolved as well.

A multicellular body's organization cannot be maintained without severely limiting cell proliferation—so that only certain cells divide, and only at appropriate times. The way cells inhibit individual growth of other cells is apparent in mammalian cells growing in tissue culture: A single layer of cells expands over a culture plate until the growing border of cells comes into contact with neighboring cells, and then the cells stop dividing. If a sector of cells is cleared away, neighboring cells rapidly refill that sector and then stop dividing again on cell contact.

How are cells able to sense the density of the cell culture around them? When cells come in contact with one another, receptor proteins in the plasma membrane activate a signal transduction pathway that acts to inhibit Cdk action. This prevents entry into the cell cycle.

Growth factors and the cell cycle

Growth factors act by triggering intracellular signaling systems. Fibroblasts, for example, possess numerous receptors on their plasma membranes for one of the first growth factors to be identified, **platelet-derived growth factor (PDGF).** The PDGF receptor is a receptor tyrosine kinase (RTK) that initiates a MAP kinase cascade to stimulate cell division (discussed in chapter 9).

PDGF was discovered when investigators found that fibroblasts would grow and divide in tissue culture only if the growth medium contained blood serum. Serum is the liquid that remains in blood after clotting; blood plasma, the liquid from which cells have been removed without clotting, would not work. The researchers hypothesized that platelets in the blood clots were releasing into the serum one or more factors required for fibroblast growth. Eventually, they isolated such a factor and named it PDGF.

Growth factors such as PDGF can override cellular controls that otherwise inhibit cell division. When a tissue is injured, a blood clot forms, and the release of PDGF triggers neighboring cells to divide, helping to heal the wound. Only a tiny amount of PDGF (approximately 10^{-10} M) is required to stimulate cell division in cells with PDGF receptors.

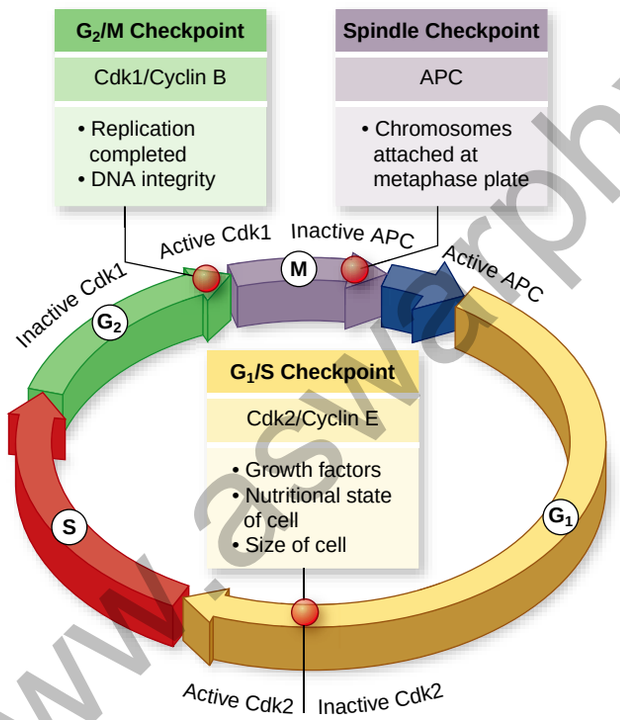


Figure 10.21 Checkpoints of the mammalian cell cycle. The more complex mammalian cell cycle is shown. This cycle is still controlled through three main checkpoints. These integrate internal and external signals to control progress through the cycle. These inputs control the state of two different Cdk–cyclin complexes and the anaphase-promoting complex (APC).

Characteristics of growth factors

Over 50 different proteins that function as growth factors have been isolated, and more undoubtedly exist. A specific cell surface receptor recognizes each growth factor, its binding site fitting that growth factor precisely. These growth factor receptors often initiate MAP kinase cascades in which the final kinase enters the nucleus and activates transcription factors by phosphorylation. These transcription factors stimulate the production of G_1 cyclins and the proteins that are necessary for cell cycle progression (figure 10.22).

The cellular selectivity of a particular growth factor depends on which target cells bear its unique receptor. Some growth factors, such as PDGF and epidermal growth factor (EGF), affect a broad range of cell types, but others affect only specific types. For example, nerve growth factor (NGF) promotes the growth of certain classes of neurons, and erythropoietin triggers cell division in red blood cell precursors. Most animal cells need a combination of several different growth factors to overcome the various controls that inhibit cell division.

The G_0 phase

If cells are deprived of appropriate growth factors, they stop at the G_1 checkpoint of the cell cycle. With their growth and division arrested, they remain in this dormant G_0 phase.

The ability to enter G_0 accounts for the incredible diversity seen in the length of the cell cycle in different tissues. Epithelial cells lining the human gut divide more than twice a day, constantly renewing this lining. By contrast, liver cells divide only once every year or two, spending most of their time in the G_0 phase. Mature neurons and muscle cells usually never leave G_0.

Cancer is a failure of cell cycle control

The unrestrained, uncontrolled growth of cells in humans leads to the disease called **cancer.** Cancer is essentially a disease of cell division—a failure of cell division control.

The p53 gene

Recent work has identified one of the culprits in cancer. Officially dubbed ***p53,*** this gene plays a key role in the G_1 checkpoint of cell division.

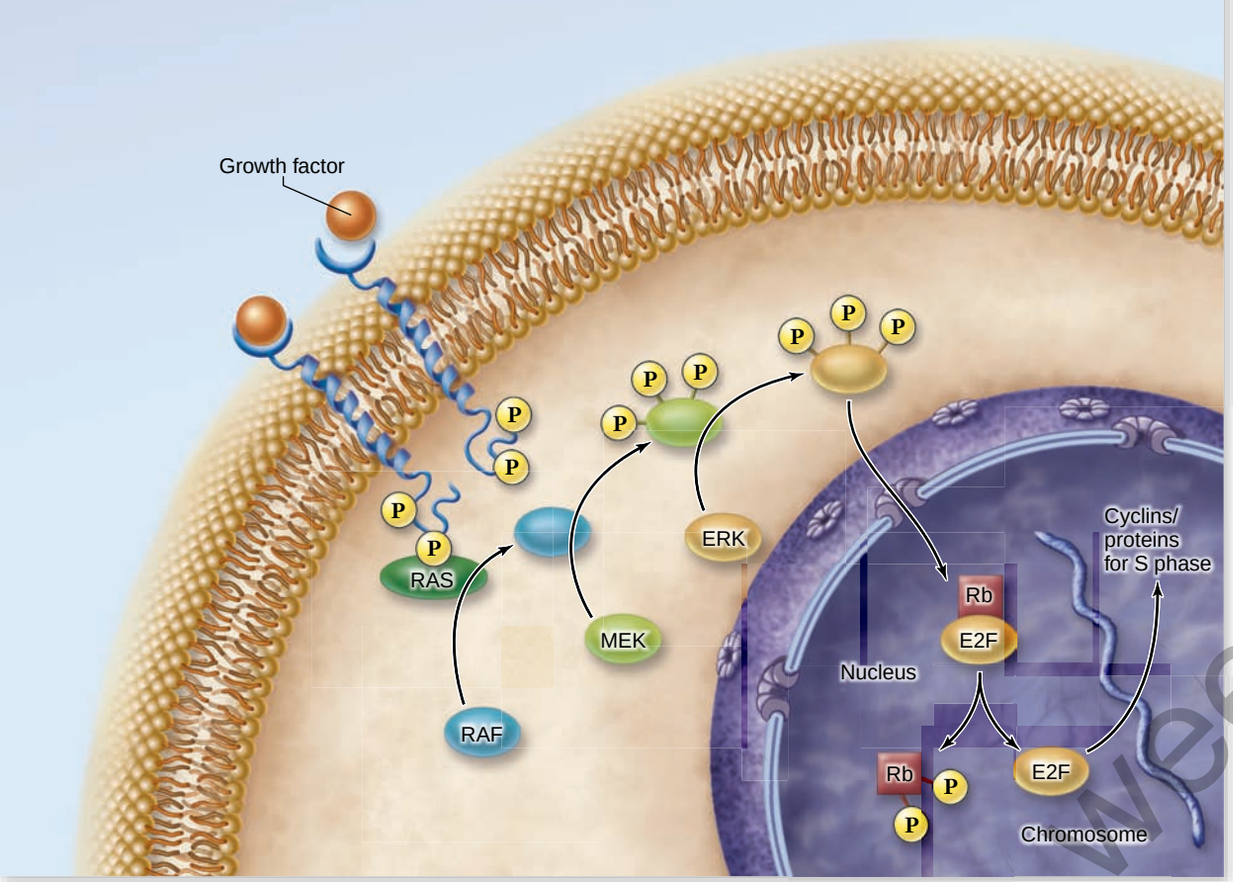


Figure 10.22 The cell proliferation-signaling pathway. Binding of a growth factor sets in motion a MAP kinase intracellular signaling pathway (described in chapter 9), which activates nuclear regulatory proteins that trigger cell division. In this example, when the nuclear retinoblastoma protein (Rb) is phosphorylated, another nuclear protein (the transcription factor E2F) is released and is then able to stimulate the production of cyclin and other proteins necessary for S phase.

The gene's product, the p53 protein, monitors the integrity of DNA, checking that it is undamaged. If the p53 protein detects damaged DNA, it halts cell division and stimulates the activity of special enzymes to repair the damage. Once the DNA has been repaired, p53 allows cell division to continue. In cases where the DNA damage is irreparable, p53 then directs the cell to kill itself.

By halting division in damaged cells, the *p53* gene prevents the development of many mutated cells, and it is therefore considered a **tumor-suppressor gene** although its activities are not limited to cancer prevention. Scientists have found that *p53* is entirely absent or damaged beyond use in the majority of cancerous cells they have examined. It is precisely because *p53* is nonfunctional that cancer cells are able to repeatedly undergo cell division without being halted at the G_1 checkpoint (figure 10.23).

Proto-oncogenes

The disease we call cancer is actually many different diseases, depending on the tissue affected. The common theme in all cases is the loss of control over the cell cycle. Research has identified numerous so-called **oncogenes,** genes that can, when introduced into a cell, cause it to become a cancer cell. This identification then led to the discovery of **proto-oncogenes,** which are normal cellular genes that become oncogenes when mutated.

The action of proto-oncogenes is often related to signalling by growth factors, and their mutation can lead to loss of growth control in multiple ways. Some proto-oncogenes encode receptors for growth factors, and others encode proteins involved in signal transduction that act after growth factor receptors. If a receptor for a growth factor becomes mutated such that it is permanently "on," the cell is no longer dependent on the presence of the growth factor for cell division. This is analogous

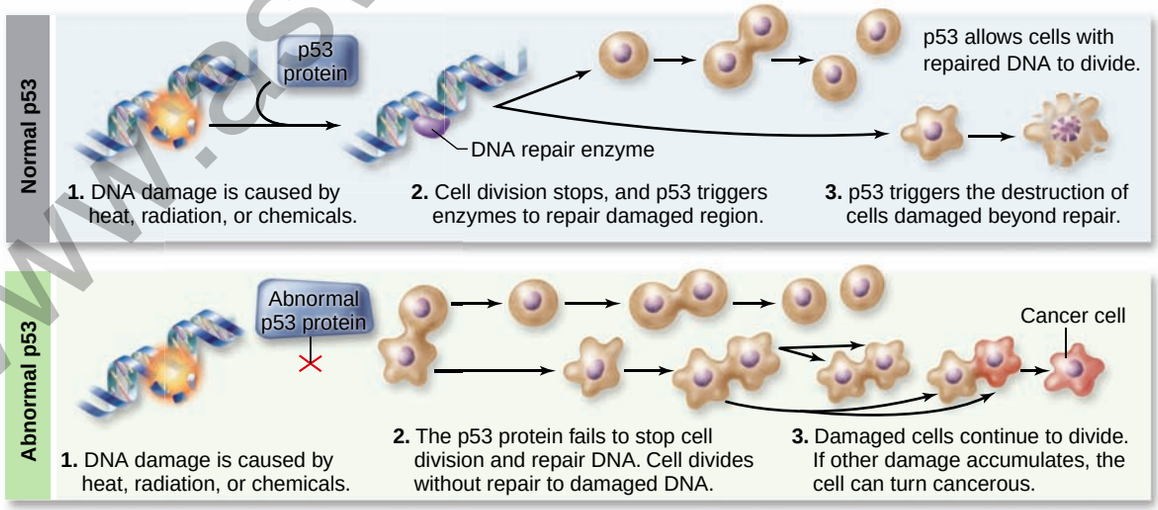


Figure 10.23 Cell division, cancer, and p53 protein. Normal p53 protein monitors DNA, destroying cells that have irreparable damage to their DNA. Abnormal p53 protein fails to stop cell division and repair DNA. As damaged cells proliferate, cancer develops.

to a light switch that is stuck on: The light will always be on. PDGF and EGF receptors both fall into the category of proto-oncogenes. Only one copy of a proto-oncogene needs to undergo this mutation for uncontrolled division to take place; thus, this change acts like a dominant mutation.

The number of proto-oncogenes identified has grown to more than 50 over the years. This line of research connects our understanding of cancer with our understanding of the molecular mechanisms governing cell cycle control.

Tumor-suppressor genes

After the discovery of proto-oncogenes, a second category of genes related to cancer was identified: the tumor-suppressor genes. We mentioned earlier that the *p53* gene acts as a tumor-suppressor gene, and a number of other such genes exist.

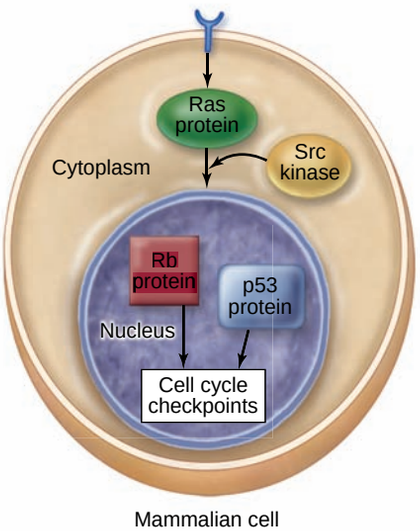
Both copies of a tumor-suppressor gene must lose function for the cancerous phenotype to develop, in contrast to the mutations in proto-oncogenes. Put another way, the proto-oncogenes act in a dominant fashion, and tumor suppressors act in a recessive fashion.

The first tumor-suppressor identified was the **retinoblastoma susceptibility gene *(Rb)***, which predisposes individuals for a rare form of cancer that affects the retina of the eye. Despite the fact that a cell heterozygous for a mutant *Rb* allele is normal, it is inherited as a dominant in families. The reason is that inheriting a single mutant copy of *Rb* means the individual has only one "good" copy left, and during the hundreds of thousands of divisions that occur to produce the retina, any error that damages the remaining good copy leads to a cancerous cell. A single cancerous cell in the retina then leads to the formation of a retinoblastoma tumor.

The role of the Rb protein in the cell cycle is to integrate signals from growth factors. The Rb protein is called a "pocket protein" because it has binding pockets for other proteins. Its role is therefore to bind important regulatory proteins and prevent them from stimulating the production of the necessary cell cycle proteins, such as cyclins or Cdks (see figure 10.21) discussed previously.

The binding of Rb to other proteins is controlled by phosphorylation: When it is dephosphorylated, it can bind a variety of regulatory proteins, but loses this capacity when phosphorylated. The action of growth factors results in the phosphorylation of Rb protein by a Cdk. This then brings us full circle, because the phosphorylation of Rb releases previously bound regulatory proteins, resulting in the production of S phase cyclins that are necessary for the cell to pass the G_1/S boundary and begin chromosome replication.

Figure 10.24 summarizes the types of genes that can cause cancer when mutated.



Proto-oncogenes

Growth factor receptor: more per cell in many breast cancers.

Ras protein: activated by mutations in 20–30% of all cancers.

Src kinase: activated by mutations in 2–5% of all cancers.

Tumor-suppressor Genes

Rb protein: mutated in 40% of all cancers.

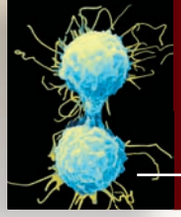
p53 protein: mutated in 50% of all cancers.

Figure 10.24 Key proteins associated with human cancers. Mutations in genes encoding key components of the cell division-signaling pathway are responsible for many cancers. Among them are proto-oncogenes encoding growth factor receptors, protein relay switches such as Ras protein, and kinase enzymes such as Src, which act after Ras and growth factor receptors. Mutations that disrupt tumor-suppressor proteins, such as Rb and p53, also foster cancer development.

Learning Outcomes Review 10.6

Cyclin proteins are produced in synchrony with the cell cycle. These proteins complex with cyclin-dependent kinases to drive the cell cyle. Three checkpoints exist in the cell cycle: the G_1/S checkpoint, the G_2/M checkpoint, and the spindle checkpoint. The cell cycle can be halted at these checkpoints if the process is not accurate. The anaphase-promoting complex/cyclosome (APC/C) triggers anaphase by lifting inhibition on a protease that removes cohesin holding chromatids together. The loss of cell cycle control leads to cancer, which can occur by a combination of two basic mechanisms: proto-oncogenes that gain function to become oncogenes, and tumor-suppressor genes that lose function and allow cell proliferation.

- ***How can you distinguish between a tumor suppressor gene and a proto-oncogene?***



Chapter Review

10.1 Bacterial Cell Division

Binary fission is a simple form of cell division.

Prokaryotic cell division is clonal, resulting in two identical cells. Bacterial DNA replication and partitioning of the chromosome are concerted processes.

Proteins control chromosome separation and septum formation.

DNA replication begins at a specific point, the origin, and proceeds bidirectionally to a specific termination site. Newly replicated chromosomes are segregated to opposite poles at the same time as they are replicated. New cells are separated by septation, which involves insertion of new cell membrane and other cellular materials at the midpoint of the cell. A ring of FtsZ and proteins embedded in the cell membrane expands radially inward, pinching the cell into two new cells.

10.2 Eukaryotic Chromosomes

Chromosome number varies among species.

The gain or loss of chromosomes is usually lethal.

Eukaryotic chromosomes exhibit complex structure.

Chromosomes are composed of chromatin, a complex of DNA, and protein. Heterochromatin is not expressed and euchromatin is expressed. The DNA of a single chromosome is a very long, double-stranded fiber. The DNA is wrapped around a core of eight histones to form a nucleosome, which can be further coiled into a 30-nm fiber in interphase cells. During mitosis, chromosomes are further condensed by arranging coiled 30-nm fibers radially around a protein scaffold.

Newly replicated chromosomes remain attached at a constricted area called a centromere, consisting of repeated DNA sequences. After replication, a chromosome consists of two sister chromatids held together at the centromere by a complex of proteins called cohesins (figure 10.7).

10.3 Overview of the Eukaryotic Cell Cycle (figure 10.8)

The cell cycle is divided into five phases.

The phases of the cell cycle are gap 1 (G_1), synthesis (S), gap 2 (G_2), mitosis, and cytokinesis (C). G_1, S, and G_2 are collectively called interphase, and mitosis and cytokinesis together are called M phase.

The duration of the cell cycle varies depending on cell type.

The length of a cell cycle varies with age, cell type, and species. Cells can exit G_1 and enter a nondividing phase called G_0; the G_0 phase can be temporary or permanent.

10.4 Interphase: Preparation for Mitosis

G_1, S, and G_2, are the three subphases of interphase. G_1 is the primary growth phase; during S phase, DNA synthesis occurs. G_2 phase occurs after S phase and before mitosis.

The centromere binds proteins assembled into a disklike structure called a kinetochore where microtubules attach during mitosis. The centromeric DNA is replicated, but the two DNA strands are held together by cohesin proteins.

10.5 M Phase: Chromosome Segregation and the Division of Cytoplasmic Contents (figure 10.11)

During prophase, the mitotic apparatus forms.

In prophase, chromosomes condense, the spindle is formed, and the nuclear envelope disintegrates. In animals cells, centriole pairs separate and migrate to opposite ends of the cell, establishing the axis of nuclear division.

During prometaphase, chromosomes attach to the spindle.

In metaphase, chromosomes align at the equator.

Chromatids of each chromosome are connected to opposite poles by kinetochore microtubules. They are held at the equator of the cell by the tension of being pulled toward opposite poles.

At anaphase, the chromatids separate.

At this point, cohesin proteins holding sister chromatids together at the centromeres are destroyed, and the chromatids are pulled to opposite poles. This movement is called anaphase A, and the movement of poles farther apart is called anaphase B.

During telophase, the nucleus re-forms.

Telophase reverses the events of prophase and prepares the cell for cytokinesis.

In animals cells, a belt of actin pinches off the daughter cells.

A contractile ring of actin under the membrane contracts during cytokinesis.

In plant cells, a cell plate divides the daughter cells.

Fusion of vesicles produces a new membrane in the middle of the cell to produce the cell plate.

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

10.6 Control of the Cell Cycle (figure 10.18)

Research uncovered cell cycle control factors.

Experiments showed that there are positive regulators of mitosis, and that there are proteins produced in synchrony with the cell cycle (cyclins). The positive regulators are cyclin-dependent kinases (Cdks). Cdks are complexes of a kinase and a regulatory molecule called cyclin. They phosphorylate proteins to drive the cell cycle.

The cell cycle can be halted at three checkpoints.

Checkpoints are points at which the cell can assess the accuracy of the process and stop if needed. The G_1/S checkpoint is a commitment to divide; the G_2/M checkpoint ensures DNA integrity; and the spindle checkpoint ensures that all chromosomes are attached to spindle fibers, with bipolar orientation.

Cyclin-dependent kinases drive the cell cycle.

The cycle progresses by the action of Cdks. Yeast have only one CDK enzyme; vertebrates have more than four enzymes. During the G_1 phase, G_1 cyclin combines with Cdc2 kinase to form the Cdk that triggers entry into S phase.

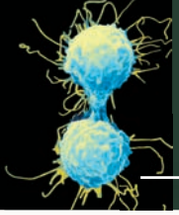
The anaphase-promoting complex/cyclosome (APC/C) activates a protease that removes cohesins holding the centromeres of sister chromatids together; the result is to trigger anaphase, separating the chromatids and drawing them to opposite poles. The APC/C also triggers destruction of mitotic cyclins to exit mitosis.

In multicellular eukaryotes, many Cdks and external signals act on the cell cycle.

Growth factors, like platelet-derived growth factor (PDGF), stimulate cell division. This acts through a MAP kinase cascade that results in the production of cyclins and activation of Cdks to stimulate cell division in fibroblasts after tissue injury.

Cancer is a failure of cell cycle control.

Mutations in proto-oncogenes have dominant, gain-of-function effects leading to cancer. Mutations in tumor-suppressor genes are recessive; loss of function of both copies leads to cancer.



Review Questions

UNDERSTAND

1. Binary fission in prokaryotes does not require the
   a. replication of DNA.
   b. elongation of the cell.
   c. separation of daughter cells by septum formation.
   d. assembly of the nuclear envelope.
2. Chromatin is composed of
   a. RNA and protein.
   b. DNA and protein.
   c. sister chromatids.
   d. chromosomes.
3. What is a nucleosome?
   a. A region in the cell's nucleus that contains euchromatin
   b. A region of DNA wound around histone proteins
   c. A region of a chromosome made up of multiple loops of chromatin
   d. A 30-nm fiber found in chromatin
4. What is the role of cohesin proteins in cell division?
   a. They organize the DNA of the chromosomes into highly condensed structures.
   b. They hold the DNA of the sister chromatids together.
   c. They help the cell divide into two daughter cells.
   d. They connect microtubules and chromosomes.
5. The kinetochore is a structure that functions to
   a. connect the centromere to microtubules.
   b. connect centrioles to microtubules.
   c. aid in chromosome condensation.
   d. aid in chromosomes cohesion.
6. Separation of the sister chromatids occurs during
   a. prophase.
   b. prometaphase.
   c. anaphase.
   d. telophase.
7. Why is cytokinesis an important part of cell division?
   a. It is responsible for the proper separation of genetic information.
   b. It is responsible for the proper separation of the cytoplasmic contents.
   c. It triggers the movement of a cell through the cell cycle.
   d. It allows cells to halt at checkpoints.

APPLY

1. What steps in the cell cycle represent irreversible commitments?
   a. The S/G_2 checkpoint
   b. The G_1/S checkpoint
   c. Anaphase
   d. Both b and c
2. Cyclin-dependent kinases (Cdks) are regulated by
   a. the periodic destruction of cyclins.
   b. bipolar attachment of chromosomes to the spindle.
   c. DNA synthesis.
   d. both a and b.
3. The bacterial SMC proteins, eukaryotic cohesin proteins, and condensin proteins share a similar structure. Functionally they all
   a. interact with microtubules.
   b. can act as kinase enzymes.
   c. interact with DNA to compact or hold strands together.
   d. connect chromosomes to cytoskeletal elements.
4. Genetically, proto-oncogenes act in a dominant fashion. This is because
   a. there is only one copy of each proto-oncogene in the genome.
   b. they act in a gain-of-function fashion to turn on the cell cycle.
   c. they act in a loss-of-function fashion to turn off the cell cycle.
   d. they require that both genomic copies are altered to affect function.
5. The metaphase to anaphase transition involves
   a. new force being generated to pull the chromatids apart.
   b. an increase in force on sister chromatids to pull them apart.
   c. completing DNA replication of centromeres allowing chromosomes to be pulled apart.
   d. loss of cohesion between sister chromatids.
6. The main difference between bacterial cell division and eukaryotic cell division is that
   a. since bacteria only have one chromosome, they can count the number of copies in the cell.
   b. eukaryotes mark their chromosomes to identify them and bacteria do not.
   c. bacterial DNA replication and chromosome segregation are concerted processes but in eukaryotes they are separated in time.
   d. none of the above
7. In animal cells, cytokinesis is accomplished by a contractile ring containing actin. The related process in bacteria is
   a. chromosome segregation, which also appears to use an actin-like protein.
   b. septation via a ring of FtsZ protein, which is an actin-like protein.
   c. cytokinesis, which requires formation of a cell plate via vesicular fusion.
   d. septation via a ring of FtsZ protein, which is a tubulin-like protein.

SYNTHESIZE

1. Regulation of the cell cycle is very complex and involves multiple proteins. In yeast, a complex of cdc2 and a mitotic cyclin is responsible for moving the cell past the G_2/M checkpoint. The activity of the cyclin-dependent kinase cdc2 is inhibited when it is phosphorylated by the kinase, Wee-1. What would you predict would be the phenotype of a Wee-1 mutant yeast? What other genes could be altered in a Wee-1 deficient mutant strain that would make the cells act normally?
2. Review your knowledge of signaling pathways (chapter 9). Create an outline illustrating how a growth factor (ligand) can lead to the production of a cyclin protein that would trigger S phase.
3. Compare and contrast how mutations in cellular proto-oncogenes and in tumor suppressor genes can lead to cancer cells.

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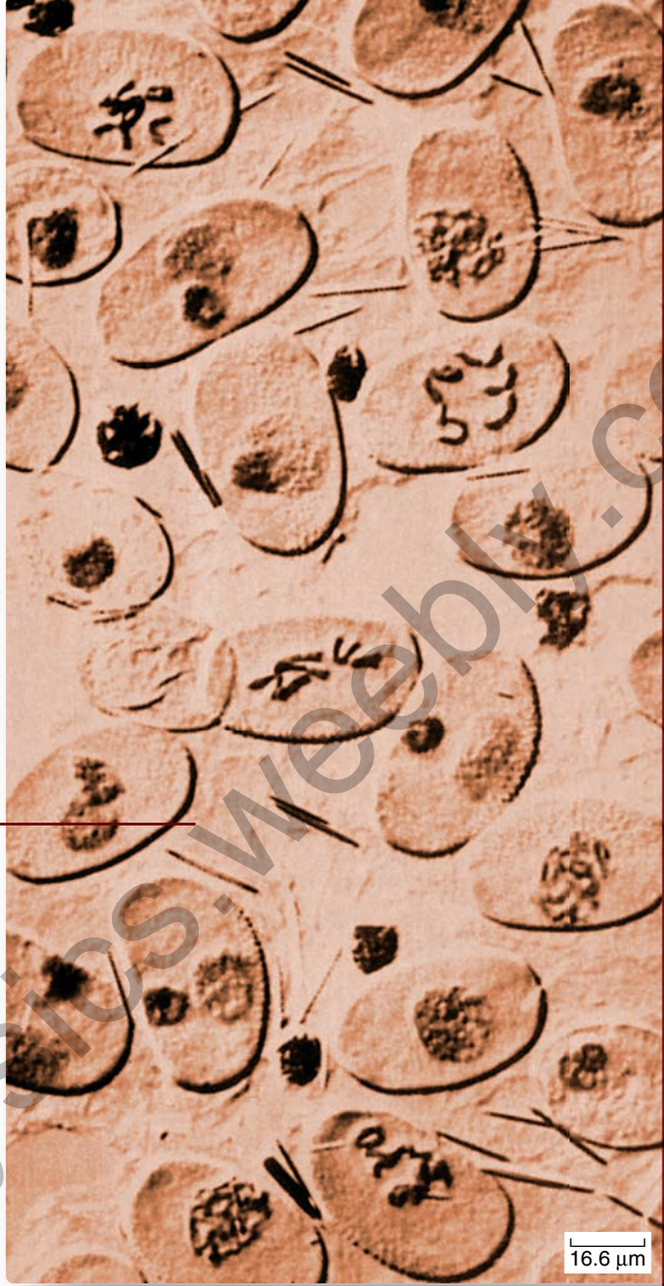
Chapter 11

Sexual Reproduction and Meiosis



Chapter Outline

Introduction

Most animals and plants reproduce sexually. Gametes of opposite sex unite to form a cell that, dividing repeatedly by mitosis, eventually gives rise to an adult body with some 100 trillion cells. The gametes that form the initial cell are the products of a special form of cell division called meiosis, *visible in the photo above, and the subject of this chapter. Meiosis is far more intricate than mitosis, and the details behind it are not as well understood. The basic process, however, is clear. Also clear are the profound consequences of sexual reproduction: It plays a key role in generating the tremendous genetic diversity that is the raw material of evolution.*

11.1 Sexual Reproduction Requires Meiosis

Learning Outcomes

1. *Understand the function of meiosis in sexual reproduction.*
2. *Distinguish between germ-line and somatic cells.*

The essence of sexual reproduction is the genetic contribution of two cells. This mode of reproduction imposes difficulties for sexually reproducing organisms that biologists recognized early on. We are only recently making progress on the underlying mechanism for the elaborate behavior of chromosomes during meiosis. To begin, we briefly consider the history of meiosis and its relationship to sexual reproduction.

Meiosis reduces the number of chromosomes

Only a few years after Walther Flemming's discovery of chromosomes in 1879, Belgian cytologist Edouard van Beneden was

surprised to find different numbers of chromosomes in different types of cells in the roundworm *Ascaris*. Specifically, he observed that the **gametes** (eggs and sperm) each contained two chromosomes, but all of the nonreproductive cells, or **somatic cells,** of embryos and mature individuals each contained four.

From his observations, van Beneden proposed in 1883 that an egg and a sperm, each containing half the complement of chromosomes found in other cells, fuse to produce a single cell called a **zygote.** The zygote, like all of the cells ultimately derived from it, contains two copies of each chromosome. The fusion of gametes to form a new cell is called **fertilization,** or **syngamy.**

It was clear even to early investigators that gamete formation must involve some mechanism that reduces the number of chromosomes to half the number found in other cells. If it did not, the chromosome number would double with each fertilization, and after only a few generations, the number of chromosomes in each cell would become impossibly large. For example, in just 10 generations, the 46 chromosomes present in human cells would increase to over 47,000 (46×2^{10}).

The number of chromosomes does not explode in this way because of a special reduction division, **meiosis.** Meiosis occurs during gamete formation, producing cells with half the normal number of chromosomes. The subsequent fusion of two of these cells ensures a consistent chromosome number from one generation to the next.

Sexual life cycles have both haploid and diploid stages

Meiosis and fertilization together constitute a cycle of reproduction. Two sets of chromosomes are present in the somatic cells of adult individuals, making them *diploid* cells, but only one set is present in the gametes, which are thus *haploid.* Reproduction that involves this alternation of meiosis and fertilization is called **sexual reproduction.** Its outstanding characteristic is that offspring inherit chromosomes from *two* parents (figure 11.1). You, for example, inherited 23 chromosomes from your mother (maternal homologue), and 23 from your father (paternal homologue).

The life cycles of all sexually reproducing organisms follow a pattern of alternation between diploid and haploid chromosome numbers, but there is some variation in the life cycles. Many types of algae, for example, spend the majority of their life cycle in a haploid state. The zygote undergoing meiosis produces haploid cells that then undergo mitosis. Some plants and some algae alternate between a multicellular haploid phase and a multicellular diploid phase (specific examples can be found in chapters 30 and 31). In most animals, the diploid state dominates; the zygote first undergoes mitosis to produce diploid cells. Then later in the life cycle, some of these diploid cells undergo meiosis to produce haploid gametes (figure 11.2).

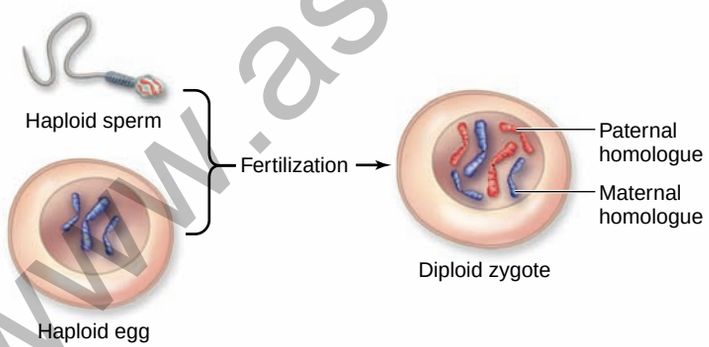


Figure 11.1 Diploid cells carry chromosomes from two parents. A diploid cell contains two versions of each chromosome, a maternal homologue contributed by the haploid egg of the mother, and a paternal homologue contributed by the haploid sperm of the father.

Germ-line cells are set aside early in animal development

In animals, the single diploid zygote undergoes mitosis to give rise to all of the cells in the adult body. The cells that will eventually undergo meiosis to produce gametes are set aside from somatic cells early in the course of development. These cells are referred to as **germ-line cells.**

Both the somatic cells and the gamete-producing germ-line cells are diploid, but whereas somatic cells undergo mitosis to form genetically identical, diploid daughter cells, gamete-producing germ-line cells undergo meiosis to produce haploid gametes (see figure 11.2).

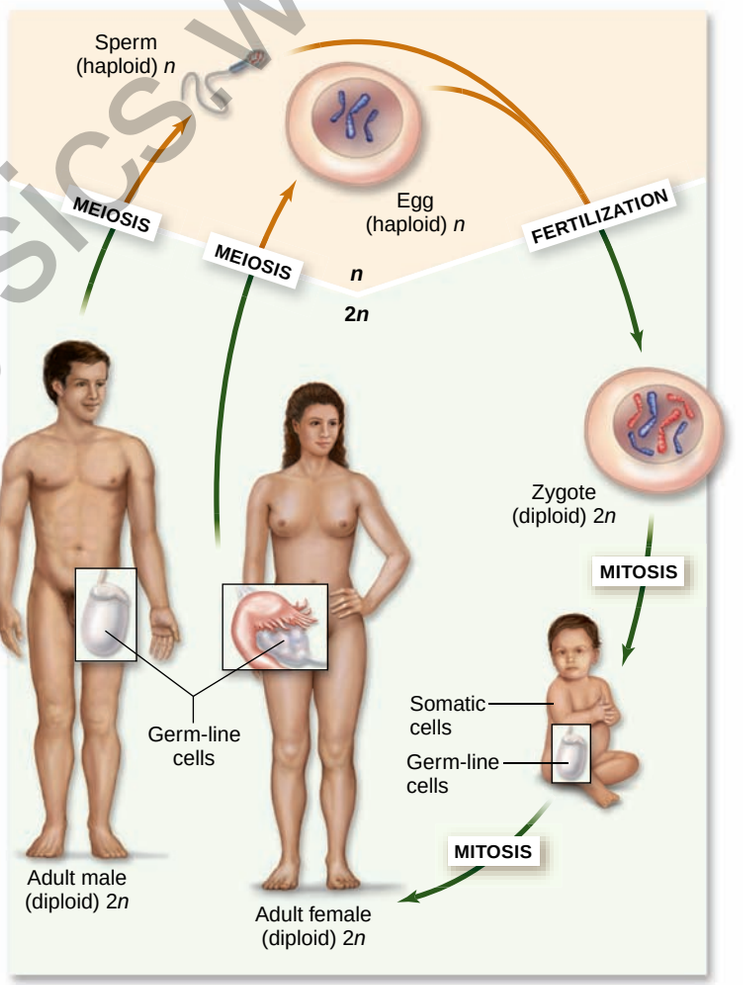


Figure 11.2 The sexual life cycle in animals. In animals, the zygote undergoes mitotic divisions and gives rise to all the cells of the adult body. Germ-line cells are set aside early in development and undergo meiosis to form the haploid gametes (eggs or sperm). The rest of the body cells are called somatic cells.

Learning Outcomes Review 11.1

Sexual reproduction involves the genetic contribution of two cells, each from a different individual. Meiosis produces haploid cells with half the number of chromosomes. Fertilization then unites these haploid cells to restore the diploid state of the next generation. Only germ-line cells are capable of meiosis. All other cells in the body, termed somatic cells, can undergo only mitotic division.

- ***Germ-line cells undergo meiosis, but how can the body maintain a constant supply of these cells?***

11.2 Features of Meiosis

Learning Outcomes

1. ***Describe how homologous chromosomes pair during meiosis.***
2. ***Explain why meiosis I is called the reductive division.***

The mechanism of meiotic cell division varies in important details in different organisms. These variations are particularly evident in the chromosomal separation mechanisms: Those found in protists and fungi are very different from those in plants and animals, which we describe here.

Meiosis in a diploid organism consists of two rounds of division, called **meiosis I** and **meiosis II,** with each round containing prophase, metaphase, anaphase, and telophase stages. Before describing the details of this process, we first examine the features of meiosis that distinguish it from mitosis.

Homologous chromosomes pair during meiosis

During early prophase I of meiosis, homologous chromosomes find each other and become closely associated, a process called pairing, or **synapsis** (figure 11.3*a*). Despite a long history of investigation, molecular details remain unclear. Biologists have used electron microscopy, data from genetic crosses, and biochemical analysis to shed light on synapsis. Thus far the results of their investigations have not been integrated into a complete picture.

The synaptonemal complex

It is clear that homologous chromosomes find their proper partners and become intimately associated during prophase I. This process includes the formation in many species of an elaborate structure called the **synaptonemal complex,** consisting of the homologues paired closely along a lattice of proteins between them (figure 11.3*b*). The components of the synaptonemal complex include a meiosis-specific form of cohesin, a type of protein that joins sister chromatids during mitosis (described in chapter 10). This form of cohesin helps to join homologues as well as sister chromatids. The result is that all four chromatids of the two homologues are closely associated during this phase of meiosis. This structure is also sometimes called a *tetrad* or *bivalent.*

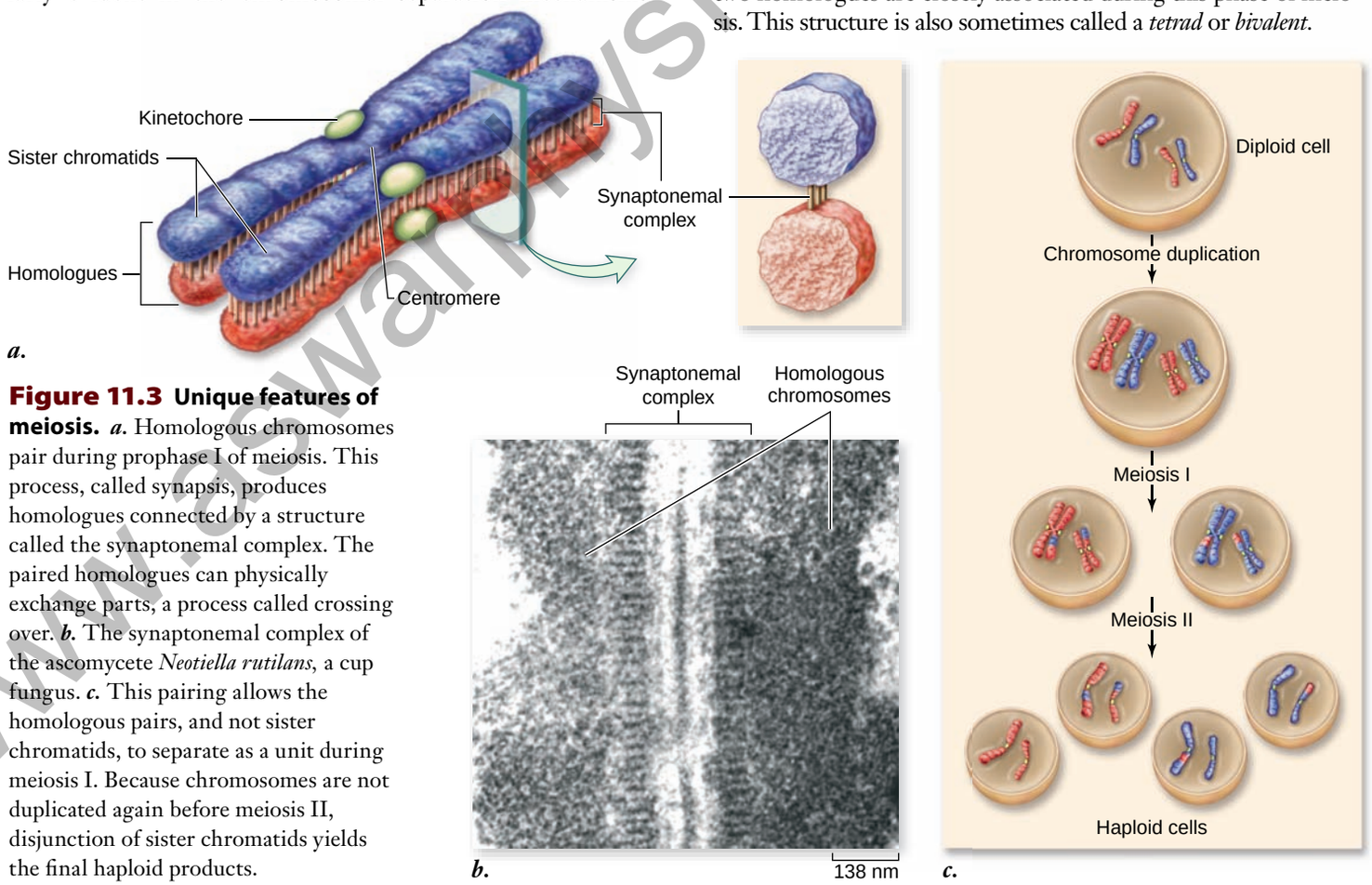


Figure 11.3 Unique features of meiosis. ***a.*** Homologous chromosomes pair during prophase I of meiosis. This process, called synapsis, produces homologues connected by a structure called the synaptonemal complex. The paired homologues can physically exchange parts, a process called crossing over. ***b.*** The synaptonemal complex of the ascomycete *Neotiella rutilans*, a cup fungus. ***c.*** This pairing allows the homologous pairs, and not sister chromatids, to separate as a unit during meiosis I. Because chromosomes are not duplicated again before meiosis II, disjunction of sister chromatids yields the final haploid products.

The exchange of genetic material between homologues

While homologues are paired during prophase I, another process unique to meiosis occurs: genetic **recombination,** or **crossing over.** This process literally allows the homologues to exchange chromosomal material. The cytological observation of this phenomenon is called crossing over, and its detection genetically is called recombination—because alleles of genes that were formerly on separate homologues can now be found on the same homologue. (Genetic recombination is covered in detail in chapter 13.)

The sites of crossing over are called **chiasmata** (singular, *chiasma*), and these sites of contact are maintained until anaphase I. The physical connection of homologues due to crossing over and the continued connection of the sister chromatids lock homologues together.

Homologue association and separation

The association between the homologues persists throughout meiosis I and dictates the behavior of the chromosomes. During metaphase I, the paired homologues move to the metaphase plate and become oriented with homologues of each pair attached to opposite poles of the spindle. By contrast, in mitosis homologues behave independently of one another.

Then, during anaphase I, homologues are pulled to opposite poles for each pair of chromosomes. This again is in contrast to mitosis, in which sister chromatids, not homologues, are pulled to opposite poles.

You can now see why the first division is termed the "reduction division"—it results in daughter cells that contain one homologue from each chromosome pair. The second meiotic division does not further reduce the number of chromosomes; it will merely separate the sister chromatids for each homologue.

Meiosis features two divisions with one round of DNA replication

The most obvious distinction between meiosis and mitosis is the simple observation that meiosis involves two successive divisions with no replication of genetic material between them. One way to view this is that DNA replication must be suppressed between the two meiotic divisions. Because of the behavior of chromosomes during meiosis I, the resulting cells contain one replicated copy of each chromosome. A division that acts like mitosis, without DNA replication, converts these cells into ones with a single copy of each chromosome (figure 11.3*c*). This is the last key to understanding meiosis: The second meiotic division is like mitosis with no chromosome duplication.

Learning Outcomes Review 11.2

Meiosis is characterized by the pairing of homologous chromosomes during prophase I. In many species, an elaborate structure called the synaptonemal complex forms between homologues. During this pairing, homologues may exchange chromosomal material at sites called chiasmata. In meiosis I, the homologues separate from each other, reducing the chromosome number to the haploid state (thus the reductive division). It is followed by a second division without replication, during which sister chromatids become separated. The result of meiosis I and II is four haploid cells.

- ***If sister chromatids separated at the first division, would meiosis still work?***

11.3 The Process of Meiosis

Learning Outcomes

1. ***Describe the behavior of chromosomes through both meiotic divisions.***
2. ***Explain the importance of monopolar attachment of homologous pairs at metaphase I.***
3. ***Differentiate between the events of anaphase I and anaphase II of meiosis.***

To understand meiosis, it is necessary to carefully follow the behavior of chromosomes during each division. The events of meiosis depend on homologues exchanging chromosomal material by crossing over. This allows sister chromatid cohesion around the sites of exchange to hold homologues together. The loss of sister chromatid cohesion is then different on the chromosome arms and at the centromeres: it is lost at anaphase I on the chromosome arms but is retained at the centromeres until anaphase II.

Prophase I sets the stage for the reductive division

Meiotic cells have an interphase period that is similar to mitosis with G_1, S, and G_2 phases. After interphase, germ-line cells enter meiosis I. In prophase I, the DNA coils tighter, and individual chromosomes first become visible under the light microscope as a matrix of fine threads. Because the DNA has already replicated before the onset of meiosis, each of these threads actually consists of two sister chromatids joined at their centromeres. In prophase I, homologous chromosomes become closely associated in synapsis, exchange segments by crossing over, and then separate.

Synapsis

During interphase in germ-line cells, the ends of the chromatids seem to be attached to the nuclear envelope at specific sites. The sites the homologues attach to are adjacent, so that during prophase I the members of each homologous pair of chromosomes are brought close together. Homologous pairs then align side by side, apparently guided by heterochromatin sequences, in the process of synapsis.

This association joins homologues along their entire length. The sister chromatids of each homologue are also joined by the cohesin complex in a process called *sister chromatid cohesion* (similar to what happens during mitosis). This brings all four chromatids for each set of paired homologues into close association.

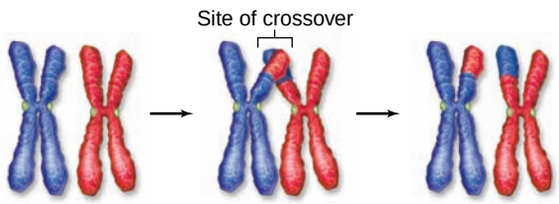


Figure 11.4 The results of crossing over. During crossing over, homologous chromosomes may exchange segments.

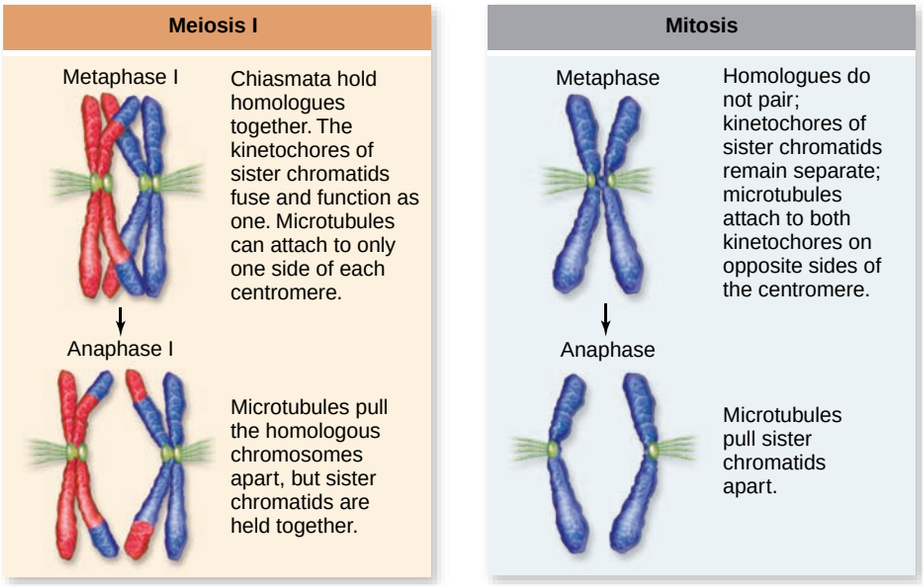


Figure 11.5 Alignment of chromosomes differs between meiosis I and mitosis. In metaphase I of meisosis I, the chiasmata and connections between sister chromatids hold homologous chromosomes together; paired kinetochores for sister chromatids of each homologue become attached to microtubules from one pole. By the end of meiosis I, connections between sister chromatid arms are broken as microtubules shorten, pulling the homologous chromosomes apart. The sister chromatids remain joined by their centromeres. In mitosis, microtubules from opposite poles attach to the kinetochore of each sister centromere; when the connections between sister centromeres are broken microtubules shorten, pulling the sister chromatids to opposite poles.

Crossing over

Along with the synaptonemal complex that forms during prophase I (see figure 11.3), another kind of structure appears that correlates in timing with the recombination process. These are called *recombination nodules*, and they are thought to contain the enzymatic machinery necessary to break and rejoin chromatids of homologous chromosomes.

Crossing over involves a complex series of events in which DNA segments are exchanged between nonsister chromatids (figure 11.4). Crossing over between sister chromatids is suppressed during meiosis. Reciprocal crossovers between nonsister chromatids are controlled such that each chromosome arm usually has one or a few crossovers per meiosis, no matter what the size of the chromosome. Human chromosomes typically have two or three.

When crossing over is complete, the synaptonemal complex breaks down, and the homologous chromosomes become less tightly associated but remain attached by chiasmata. At this point, there are four chromatids for each type of chromosome (two homologous chromosomes, each of which consists of two sister chromatids).

The four chromatids do not separate completely because they are held together in two ways: (1) The two sister chromatids of each homologue, recently created by DNA replication, are held together by their common centromeres; and (2) the paired homologues are held together at the points where crossing over occurred by sister chromatid cohesion around the site of exchange. These points are the chiasmata that can be observed microscopically. Like small rings moving down two strands of rope, the chiasmata move to the end of the chromosome arm before metaphase I.

While the elaborate behavior of chromosome pairing is taking place, other events must occur during prophase I. The nuclear envelope must be dispersed, along with the interphase structure of microtubules. The microtubules are formed into a spindle, just as in mitosis.

During metaphase I, paired homologues align

By metaphase I, the second stage of meiosis I, the chiasmata have moved down the paired chromosomes to the ends. At this point, they are called *terminal chiasmata*. Terminal chiasmata hold the homologous chromosomes together in metaphase I so that homologues can be aligned at the equator of the cell.

The capture of microtubules by kinetochores occurs such that the kinetochores of sister chromatids act as a single unit. This results in microtubules from opposite poles becoming attached to the kinetochores of *homologues*, and not to those of sister chromatids (figure 11.5).

The ability of sister centromeres to behave as a unit during meiosis I is not understood. It has been suggested, based on electron microscope data, that the centromere–kinetochore complex of sister chromatids is compacted during meiosis I, allowing them to function as a single unit.

The monopolar attachment of centromeres of sister chromatids would be disastrous in mitosis, but it is critical to meiosis I. It produces tension on the homologues, which are joined by chiasmata and sister chromatid cohesion, pulling paired homologues to the equator of the cell. In this way, each joined pair of homologues lines up on the metaphase plate (see figure 11.5).

The orientation of each pair on the spindle axis is random; either the maternal or the paternal homologue may be oriented toward a given pole (figure 11.6; see also figure 11.7).

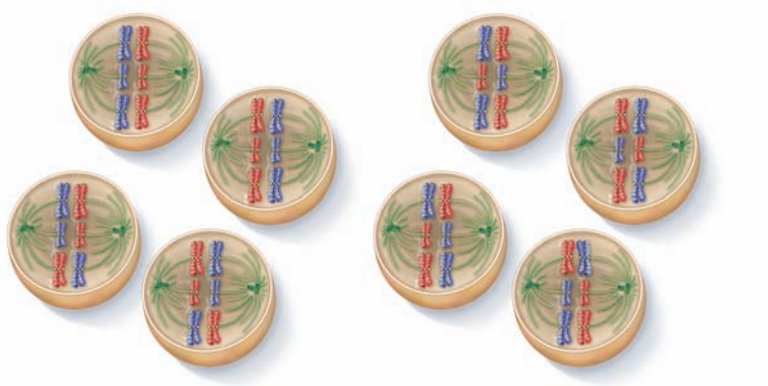
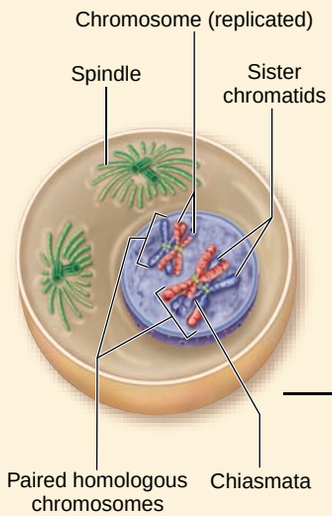


Figure 11.6 Random orientation of chromosomes on the metaphase plate. The number of possible chromosome orientations equals 2 raised to the power of the number of chromosome pairs. In this hypothetical cell with three chromosome pairs, eight (2^3) possible orientations exist. Each orientation produces gametes with different combinations of parental chromosomes.

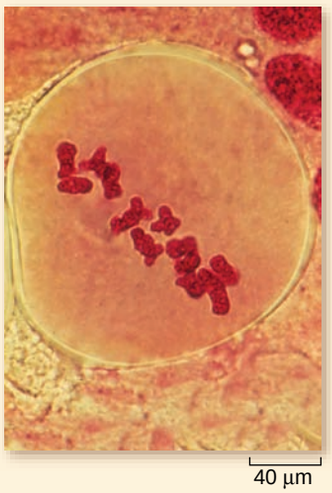
MEIOSIS I

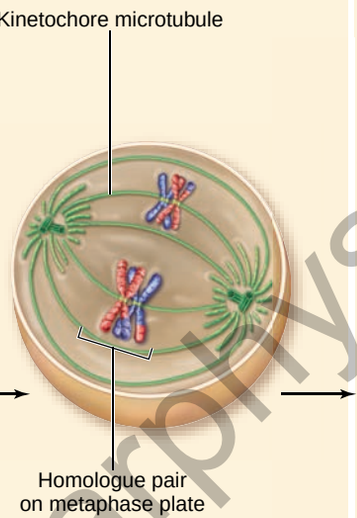
Prophase I



In prophase I of meiosis I, the chromosomes begin to condense, and the spindle of microtubules begins to form. The DNA has been replicated, and each chromosome consists of two sister chromatids attached at the centromere. In the cell illustrated here, there are four chromosomes, or two pairs of homologues. Homologous chromosomes pair up and become closely associated during synapsis. Crossing over occurs, forming chiasmata, which hold homologous chromosomes together.

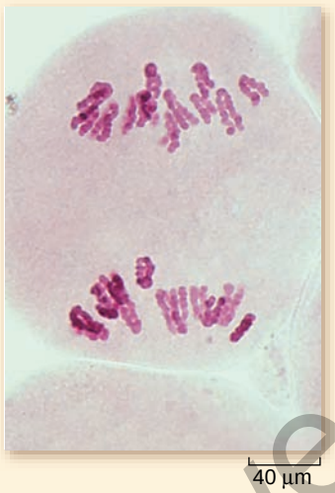
Metaphase I

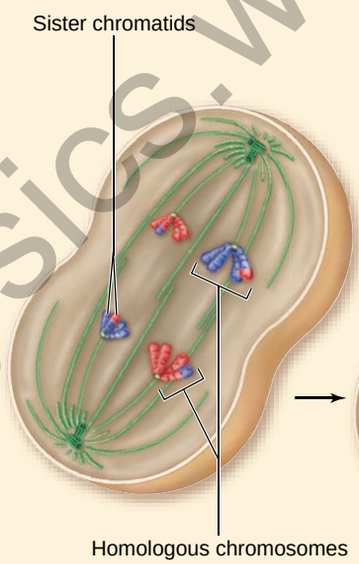




In metaphase I, the pairs of homologous chromosomes align along the metaphase plate. Chiasmata help keep the pairs together and produce tension when microtubules from opposite poles attach to sister kinetochores of each homologue. A kinetochore microtubule from one pole of the cell attaches to one homologue of a chromosome, while a kinetochore microtubule from the other cell pole attaches to the other homologue of a pair.

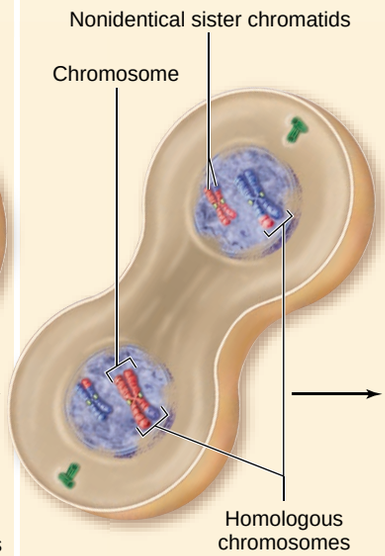
Anaphase I





In anaphase I, kinetochore microtubules shorten, and homologous pairs are pulled apart. One duplicated homologue goes to one pole of the cell, while the other duplicated homologue goes to the other pole. Sister chromatids do not separate.This is in contrast to mitosis, where duplicated homologues line up individually on the metaphase plate, kinetochore microtubules from opposite poles of the cell attach to opposite sides of one homologue's centromere, and sister chromatids are pulled apart in anaphase.

Telophase I

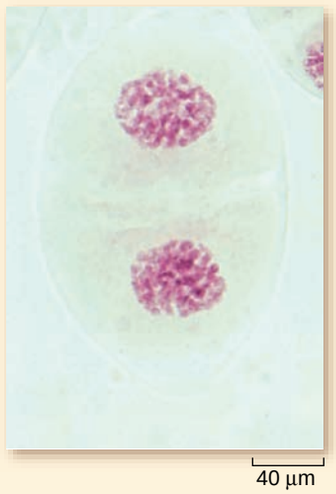


In telophase I, the separated homologues form a cluster at each pole of the cell, and the nuclear envelope re-forms around each daughter cell nucleus. Cytokinesis may occur. The resulting two cells have half the number of chromosomes as the original cell: In this example, each nucleus contains two chromosomes (versus four in the original cell). Each chromosome is still in the duplicated state and consists of two sister chromatids, but sister chromatids are not identical because crossing over has occurred.

Figure 11.7 The stages of meiosis. Meiosis in plant cells (photos) and animal cells (drawings) is shown.

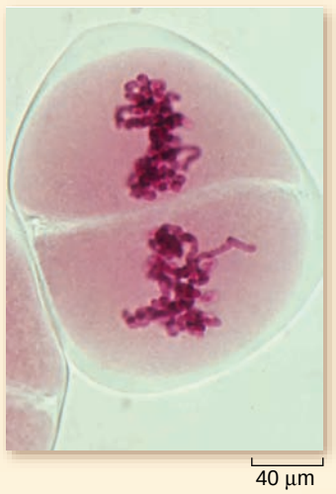
MEIOSIS II

Prophase II



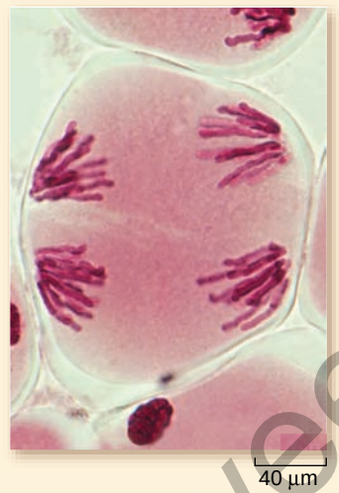
Following a typically brief interphase, with no S phase, meiosis II begins. During prophase II, a new spindle apparatus forms in each cell, and the nuclear envelope breaks down. In some species the nuclear envelope does not re-form in telophase I removing the need for nuclear envelope breakdown.

Metaphase II



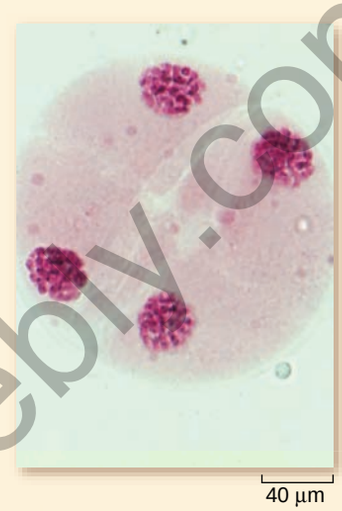
In metaphase II, a completed spindle apparatus is in place in each cell. Chromosomes consisting of sister chromatids joined at the centromere align along the metaphase plate in each cell. Now, kinetochore microtubules from opposite poles attach to kinetochores of sister chromatids.

Anaphase II



When microtubules shorten in anaphase II, the centromeres split, and sister chromatids are pulled to opposite poles of the cells.

Telophase II



In telophase II, the nuclear membranes re-form around four different clusters of chromosomes. After cytokinesis, four haploid cells result. No two cells are alike due to the random alignment of homologous pairs at metaphase I and crossing over during prophase I.

Anaphase I results from the differential loss of sister chromatid cohesion along the arms

In anaphase I, the microtubules of the spindle fibers begin to shorten. As they shorten, they break the chiasmata and pull the centromeres toward the poles, dragging the chromosomes along with them.

Anaphase I comes about by the release of sister chromatid cohesion along the chromosome arms, but not at the centromeres. This release is thought to be the result of the destruction of meiosis-specific cohesin in a process analogous to anaphase in mitosis. The difference is that the destruction is inhibited at the centromeres by a mechanism that is only recently becoming clear.

As a result of this release, the homologues are pulled apart, but not the sister chromatids. Each homologue moves to one pole, taking both sister chromatids with it. When the spindle fibers have fully contracted, each pole has a complete haploid set of chromosomes consisting of one member of each homologous pair.

Because of the random orientation of homologous chromosomes on the metaphase plate, a pole may receive either the maternal or the paternal homologue from each chromosome pair. As a result, the genes on different chromosomes assort independently; that is, meiosis I results in the **independent assortment** of maternal and paternal chromosomes into the gametes (see chapter 12).

Telophase I completes meiosis I

By the beginning of telophase I, the chromosomes have segregated into two clusters, one at each pole of the cell. Now the nuclear membrane re-forms around each daughter nucleus.

Because each chromosome within a daughter nucleus had replicated before meiosis I began, each now contains two sister chromatids attached by a common centromere. Note that *the sister chromatids are no longer identical* because of the crossing over that occurred in prophase I (see figure 11.7); as you will see, this change has important implications for genetic variability.

Cytokinesis, the division of the cytoplasm and its contents, may or may not occur after telophase I. The second meiotic division, meiosis II, occurs after an interval of variable length.

Achiasmate segregation of homologues is possible

The preceding description of meiosis I relies on the observation that homologues are held together by chiasmata and by sister chromatid cohesion. This connection produces the critical behavior of chromosomes during metaphase I and anaphase I, when homologues move to the metaphase plate and then move to opposite poles.

Although this connection of homologues is the rule, there are exceptions. In fruit fly *(Drosophila)* males for example, there is no recombination, and yet meiosis proceeds accurately, a process called **achiasmate segregation** ("without chiasmata"). This seems to involve an alternative mechanism for joining homologues and then allowing their segregation during anaphase I. Telomeres and other heterochromatic sequences have been implicated, but the details remain unclear.

Despite these exceptions, the vast majority of species that have been examined use the formation of chiasmata and sister chromatid cohesion to hold homologues together for segregation during anaphase I.

Meiosis II is like a mitotic division without DNA replication

Typically, interphase between meiosis I and meiosis II is brief and does not include an S phase: Meiosis II resembles a normal mitotic division. Prophase II, metaphase II, anaphase II, and telophase II follow in quick succession (see figure 11.7).

Prophase II. At the two poles of the cell, the clusters of chromosomes enter a brief prophase II, each nuclear envelope breaking down as a new spindle forms.

Metaphase II. In metaphase II, spindle fibers from opposite poles bind to kinetochores of each sister chromatid, allowing each chromosome to migrate to the metaphase plate as a result of tension on the chromosomes from polar microtubules pulling on sister centromeres. This process is the same as metaphase during a mitotic division.

Anaphase II. The spindle fibers contract, and the cohesin complex joining the centromeres of sister chromatids is destroyed, splitting the centromeres and pulling the sister chromatids to opposite poles. This process is also the same as anaphase during a mitotic division.

Telophase II. Finally, the nuclear envelope re-forms around the four sets of daughter chromosomes. Cytokinesis then follows.

The final result of this division is four cells containing haploid sets of chromosomes. The cells that contain these haploid nuclei may develop directly into gametes, as they do in animals. Alternatively, they may themselves divide mitotically, as they do in plants, fungi, and many protists, eventually producing greater numbers of gametes or, as in some plants and insects, adult individuals with varying numbers of chromosome sets.

Errors in meiosis produce aneuploid gametes

It is critical that the process of meiosis be accurate because any failure produces gametes without the correct number of chromosomes. Failure of chromosomes to move to opposite poles during either meiotic division is called *nondisjunction*, and it produces one gamete that lacks a chromosome and one that has two copies. Gametes with an improper number of chromosomes are called **aneuploid gametes.** In humans, this condition is the most common cause of spontaneous abortion. The implications of aneuploid gametes are explored in more detail in chapter 13.

Learning Outcomes Review 11.3

Sister chromatid cohesion, combined with crossing over, connects homologous chromosomes during meiosis I. The centromere of each homologue shows monopolar attachment, leading to the alignment of homologous pairs at metaphase I. Loss of cohesion on the arms but not the centromere leads to homologues moving to opposite poles during anaphase I. During anaphase II, cohesin proteins holding sister chromatids together at the centromere are removed, allowing them to move to opposite poles.

- ***What would be the result of improper disjunction at anaphase I? At anaphase II?***

11.4 Summing Up: Meiosis Versus Mitosis

Learning Outcomes

1. ***Understand the distinct features of meiosis.***
2. ***Describe the differences in chromatid cohesion in meiosis and mitosis.***
3. ***Explain the importance of the suppression of replication between meiotic divisions.***

The key to meiosis is understanding the differences between meiosis and mitosis. The basic machinery in both processes is the same, but the behavior of chromosomes is distinctly different during the first meiotic division (figure 11.8).

Meiosis is characterized by four distinct features:

1. Homologous pairing and crossing over joins maternal and paternal homologues during meiosis I.
2. Sister chromatids remain connected at the centromere and segregate together during anaphase I.
3. Kinetochores of sister chromatids are attached to the same pole in meiosis I and to opposite poles in mitosis.
4. DNA replication is suppressed between the two meiotic divisions.

Although the underlying molecular mechanisms are unclear, we will consider what we know of each of these features in the following sections.

Homologous pairing is specific to meiosis

The pairing of homologues during prophase I of meiosis is the first deviation from mitosis and sets the stage for all of the subsequent differences (see figure 11.8). How homologues find each other and become aligned is one of the great mysteries of meiosis. Some cytological evidence implicates telomeres and other specific sites as being necessary for pairing, but this finding does little to clarify the essential process.

Some light has been shed on the mechanisms with the discovery of meiosis-specific cohesin proteins. In yeast, the protein Rec8 replaces the mitotic Scc1 protein as part of the cohesin complex. You saw in chapter 10 that Scc1 is destroyed during anaphase of mitosis to allow sister chromatids to be pulled to opposite poles. The replacement of this critical cohesin component with a meiosis-specific version seems to be a common feature in systems analyzed to date.

Synaptonemal complex proteins have been identified in diverse species, but these proteins show little sequence conservation. This is despite the similarity of structures observed cytologically. The transverse elements, while showing no sequence conservation, do share the feature of coiled-coil domains that promote protein–protein interactions.

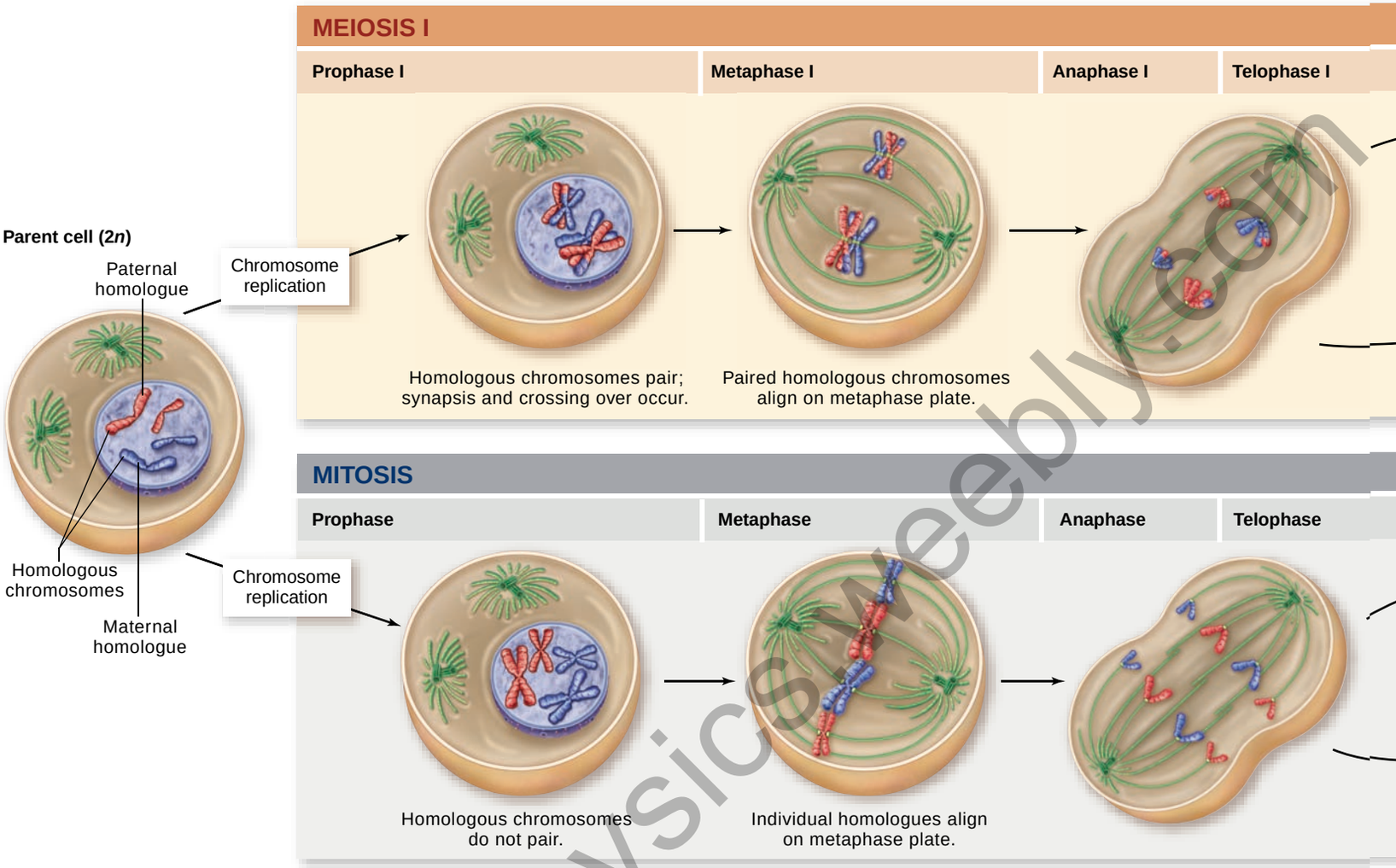
The molecular details of the recombination process that produces crossing over are complex, but many of the proteins involved have been identified. The process is initiated with the introduction of a double-strand break in one homologue. This explains the similarity in the machinery necessary for meiotic recombination and the machinery involved in the repair of double-strand breaks in DNA. Recombination probably first evolved as a repair mechanism and was later co-opted for use in disjoining chromosomes. The importance of recombination for proper disjunction is clear from the observation in many organisms that loss of function for recombination proteins also results in higher levels of nondisjunction.

Sister chromatid cohesion is maintained through meiosis I but released in meiosis II

Meiosis I is characterized by the segregation of homologues, not sister chromatids, during anaphase. For this separation to occur, the centromeres of sister chromatids must move to the same pole, or cosegregate, during anaphase I. This means that meiosis-specific cohesin proteins must first be removed from the chromosome arms, then later from sister centromeres.

Homologues are joined by chiasmata, and sister chromatid cohesion around the site of exchange then holds homologues together. The destruction of Rec8 protein on the chromosome arms appears to be what allows homologues to be pulled apart at anaphase I.

This leaves the key distinction between meiosis and mitosis being the maintenance of sister chromatid cohesion at the centromere during all of meiosis I, but the loss of cohesion from the chromosome arms during anaphase I (see figure 11.8). Recently, some light was shed on this problem with the identification of conserved proteins, called Shugoshin (a Japanese term meaning "guardian spirit") required for cohesin protection from separase-mediated cleavage during meiosis I (figure 11.9). Mice have two Shugoshins: Sgo-1 and Sgo-2. Depletion of Sgo-2 results in early sister chromatid separation. This leaves the problem of why Sgo-2 acts only at anaphase I and not anaphase II. It has been suggested that the tension produced by anaphase II causes Sgo-2 to migrate from the centromere to the kinetochore.



Sister kinetochores are attached to the same pole during meiosis I

The cosegregation of sister centromeres requires that the kinetochores of sister chromatids are attached to the same pole during meiosis I. This attachment is in contrast to both mitosis (see figure 11.8) and meiosis II, in which sister kinetochores must become attached to opposite poles.

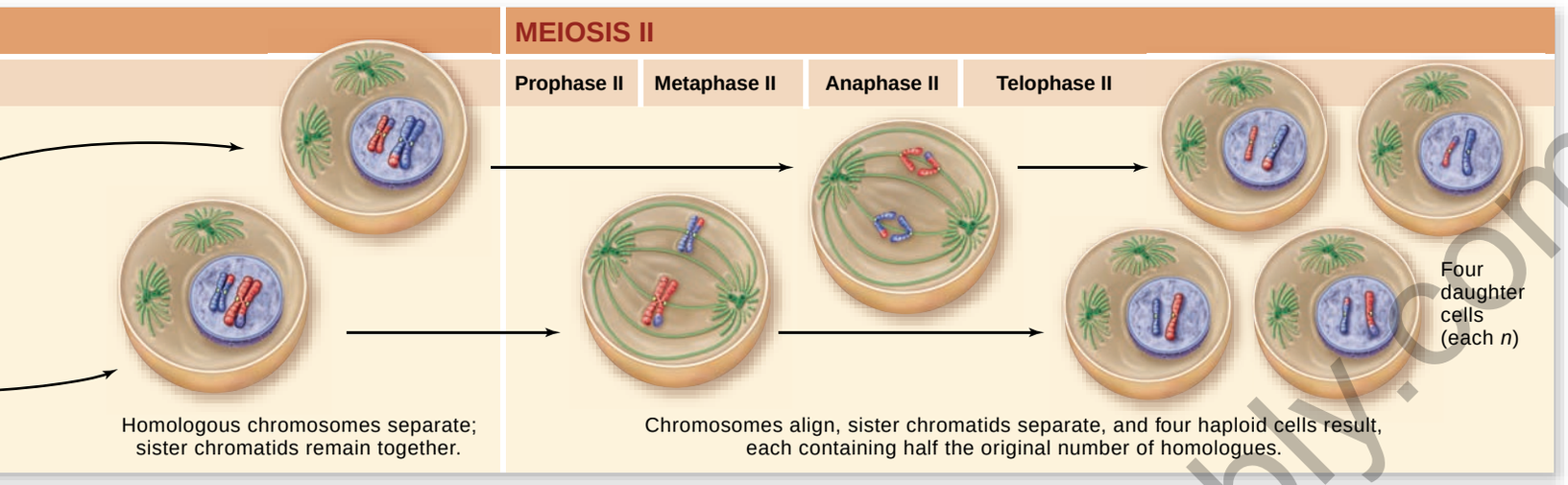
The underlying basis of this monopolar attachment of sister kinetochores is unclear, but it seems to be based on structural differences between centromere–kinetochore complexes in meiosis I and in mitosis. Mitotic kinetochores visualized with the electron microscope appear to be recessed, making bipolar attachment more likely. Meiosis I kinetochores protrude more, making monopolar attachment easier.

It is clear that both the maintenance of sister chromatid cohesion at the centromere and monopolar attachment are required for the segregation of homologues that distinguishes meiosis I from mitosis.

Replication is suppressed between meiotic divisions

After a mitotic division, a new round of DNA replication must occur before the next division. For meiosis to succeed in halving the number of chromosomes, this replication must be suppressed between the two divisions. The detailed mechanism of suppression of replication between meiotic division is unknown. One clue is the observation that the level of one of the cyclins, cyclin B, is reduced between meiotic divisions, but is not lost completely, as it is between mitotic divisions.

During mitosis, the destruction of mitotic cyclin is necessary for a cell to enter another division cycle. The result of this maintenance of cyclin B between meiotic divisions in germ-line cells is the failure to form initiation complexes necessary for DNA replication to proceed. This failure to form initiation complexes appears to be critical to suppressing DNA replication.



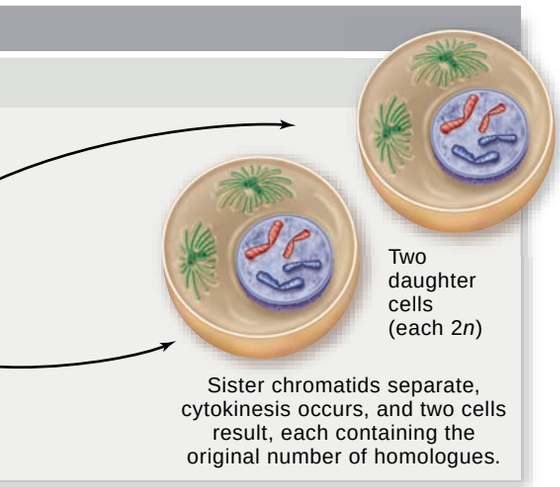


Figure 11.8 A comparison of meiosis and mitosis. Meiosis involves two nuclear divisions with no DNA replication between them. It thus produces four daughter cells, each with half the original number of chromosomes. Crossing over occurs in prophase I of meiosis. Mitosis involves a single nuclear division after DNA replication. It thus produces two daughter cells, each containing the original number of chromosomes.

Inquiry question

If the chromosomes of a mitotic cell behaved the same as chromosomes in meiosis I, would the resulting cells have the proper chromosomal constitution?

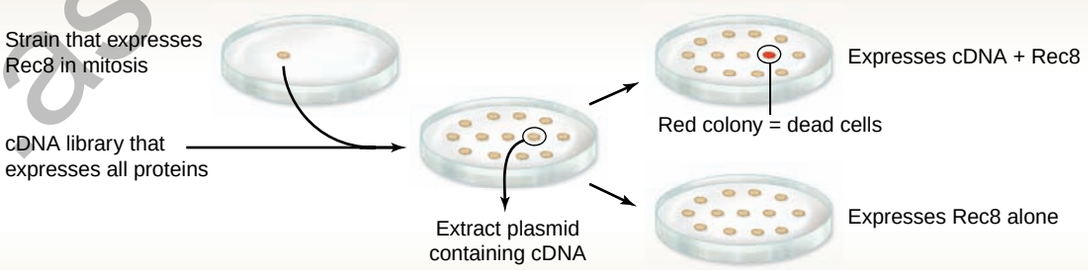
SCIENTIFIC THINKING

Question: *Why are cohesin proteins at the centromeres of sister chromatids not destroyed at anaphase I of meiosis?*

Hypothesis: *Meiosis-specific cohesin component Rec8 is protected by another protein at centromeres.*

Prediction: *If Rec8 and the centromere protecting protein are both expressed in mitotic cells, chromosome separation will be prevented. This is lethal to a dividing cell.*

Test: *Fission yeast strain is designed to produce Rec8 instead of normal mitotic cohesin. These cells are transformed with a cDNA library that expresses all cellular proteins. Transformed cells are duplicated onto media containing dye for dead cells (allows expression of Rec8 and cDNA), and media that will result in loss of plasmid cDNA (expresses only Rec8). Cells containing cDNA for protecting protein will be dead in presence of Rec8.*



Result: *Transformed cells that die on the plates where Rec8 is coexpressed with cDNA identify the protecting protein. When the cDNA is extracted and analyzed, the encoded protein localizes to the centromeres of meiotic cells.*

Conclusion: *This screen identifies a protein with Rec8 protecting activity.*

Further Experiments: *If the gene encoding the protecting protein is deleted from cells, what would be the expected phenotype? In mitotic cells? In meiotic cells?*

Figure 11.9 Identification of meiosis-specific cohesin protector.

Meiosis produces cells that are not identical

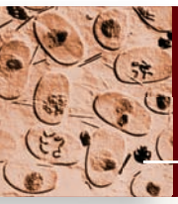
The daughter cells produced by mitosis are identical to the parental cell, at least in terms of their chromosomal constitution. This exact copying is critical to producing new cells for growth, for development, and for wound healing. Meiosis, because of the random orientation of different chromosomes at the first meiotic division and because of crossing over, rarely produces cells that are identical. The gametes from meiosis all carry an entire haploid set of chromosomes, but these chromosomes are a mixture of maternal and paternal homologues; furthermore, the homologues themselves have exchanged material by crossing over. The resulting variation is essential for evolution and is the reason that sexually reproducing populations have much greater variation than asexually reproducing ones.

Meiosis is not only critical for the process of sexual reproduction, but is also the foundation for understanding the basis of heredity. The different cells produced by meiosis form the basis for understanding the behavior of observable traits in genetic crosses. In the next two chapters we will follow the behavior of traits in genetic crosses and see how this correlates with the behavior of chromosomes in meiosis.

Learning Outcomes Review 11.4

Meiosis is characterized by homologue pairing and crossing over; by loss of sister chromatid cohesion in the arms, but not at the centromere at the first division; and by the suppression of DNA replication between the two meiotic divisions. Sister chromatid cohesion would be disastrous in mitosis, but in meiosis I it allows the reduction of chromosome number. If replication were not suppressed between meiosis I and meiosis II, gametes would be diploid, and zygotes would be tetraploid.

- ***What features of meiosis lead to genetic variation in the products?***



Chapter Review

11.1 Sexual Reproduction Requires Meiosis (figure 11.2)

Meiosis reduces the number of chromosomes.

Eggs and sperm are haploid ($1n$) cells, which contain one set of all chromosomes, and products of meiotic division.

Sexual life cycles have both haploid and diploid stages.

During fertilization, or syngamy, the fusion of two haploid gametes results in a diploid ($2n$) zygote, which contains two sets of chromosomes. Meiosis and fertilization constitute a reproductive cycle in sexual organisms as they alternate between diploid and haploid chromosome numbers. Somatic cells divide by mitosis and form the body of an organism.

Germ-line cells are set aside early in animal development.

Cells that eventually will form haploid gametes by meiosis are called germ-line cells. These are set aside early in development in animals.

11.2 Features of Meiosis

Homologous chromosomes pair during meiosis.

The pairing of homologous chromosomes, called synapsis, occurs during early prophase I. Paired homologues are often joined by the synaptonemal complex (see figure 11.3). During synapsis, crossing over occurs between homologous chromosomes, exchanging chromosomal material (see figure 11.4). Because the homologues are paired, they move as a unit to the metaphase plate during metaphase I. During anaphase I, homologues of each pair are pulled to opposite poles, producing two cells that each have one complete set of chromosomes.

Meiosis features two divisions with one round of DNA replication.

Meiosis II is like mitosis but without replication of DNA. Sister chromatids are pulled to opposite poles to yield four haploid cells.

11.3 The Process of Meiosis (see figures 11.7 and 11.8)

Prophase I sets the stage for the reductive division.

Meiotic cells have an interphase period similar to mitosis with G_1, S, and G_2 phases. This is followed by prophase I in which homologous chromosomes align along their entire length. The sister chromatids are held together by cohesin proteins. Homologues exchange chromosomal material by crossing over, which assists in holding the homologues together during meiosis I. The nuclear envelope disperses and the spindle apparatus forms.

During metaphase I, paired homologues align.

Spindle fibers attach to the kinetochores of the homologues; the kinetochores of sister chromatids behave as a single unit. Homologues of each pair become attached by kinetochore microtubules to opposite poles, and homologous pairs move to the metaphase plate as a unit. The orientation of each homologous pair on the equator is random; either the maternal or paternal homologue may be oriented toward a given pole.

Anaphase I results from the differential loss of sister chromatid cohesion along the arms.

During anaphase I the homologues of each pair are pulled to opposite poles as kinetochore microtubules shorten. Loss of sister chromatid cohesion on the arms but not at the centromeres allows homologues to separate, but sister chromatids to stay together. This is due to the loss of cohesin proteins on the arms but not at the centromere. At the end of anaphase I each pole has a complete set of haploid chromosomes, consisting of one member of each homologous pair. Because of the random orientation of homologous pairs at metaphase I, meiosis I results in the independent assortment of maternal and paternal chromosomes in gametes.

Telophase I completes meiosis I.

During telophase I the nuclear envelope re-forms around each daughter nucleus. This phase does not occur in all species. Cytokinesis may or may not occur after telophase I.

Achiasmate segregation of homologues is possible.

Although homologues are usually held together by chiasmata, some systems are able to segregate chromosomes without this.

Meiosis II is like a mitotic division without DNA replication.

A brief interphase with no DNA replication occurs after meiosis I. During meiosis II, cohesin proteins at the centromeres that hold sister chromatids together are destroyed, allowing each to migrate to opposite poles of the cell. The result of meiosis I and II is four cells, each containing haploid sets of chromosomes that are not identical. Once completed, the haploid cells may produce gametes or divide mitotically to produce even more gametes or haploid adults.

Errors in meiosis produce aneuploid gametes.

Errors occur during meiosis because of nondisjunction, the failure of chromosomes to move to opposite poles. It may result in aneuploid gametes: one gamete with no chromosome, and another gamete with two copies of a chromosome.

11.4 Summing Up: Meiosis Versus Mitosis

Four distinct features of meiosis I are not found in mitosis: Maternal and paternal homologues pair, and exchange genetic information by crossing over; the kinetochores of sister chromatids function as a unit during meiosis I, allowing sister chromatids to cosegregate during anaphase I; kinetochores of sister chromatids are connected to a single pole in meiosis I and to opposite poles in mitosis; and DNA replication is suppressed between meiosis I and meiosis II.

Homologous pairing is specific to meiosis.

How homologues find each other during meiosis is not known. The proteins of the synaptonemal complex do not seem to be conserved in different species, but there are meiosis-specific cohesin proteins. These are involved in the differential destruction of cohesins on the arms versus the centromere during meiosis I. The recombination process that occurs between paired homologues is better known. This process uses proteins involved in DNA repair and starts with a double-stranded break in DNA.

Sister chromatid cohesion is maintained through meiosis I but released in meiosis II.

Shugoshin protein protects centromeric cohesin in anaphase I, so that sister chromatids remain connected. Cohesins on the arms are not protected and are thus degraded during anaphase I, allowing homologues to move to opposite poles.

Sister kinetochores are attached to the same pole during meiosis I.

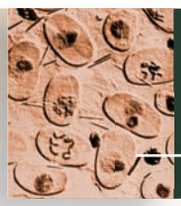
Kinetochores of sister chromatids must be attached to the same spindle fibers (monopolar attachment) to segregate together.

Replication is suppressed between meiotic divisions.

Suppression of replication may be related to the maintenance of some cyclin proteins that are degraded at the end of mitosis.

Meiosis produces cells that are not identical.

Because of the independent assortment of homologues and the process of crossing over, gametes show great variation.



Review Questions

UNDERSTAND

1. In comparing somatic cells and gametes, somatic cells are
   a. diploid with half the number of chromosomes.
   b. haploid with half the number of chromosomes.
   c. diploid with twice the number of chromosomes.
   d. haploid with twice the number of chromosomes.
2. What are *homologous* chromosomes?
   a. The two halves of a replicated chromosome
   b. Two identical chromosomes from one parent
   c. Two genetically identical chromosomes, one from each parent
   d. Two genetically similar chromosomes, one from each parent
3. Chiasmata form
   a. between homologous chromosomes.
   b. sister chromatids.
   c. between replicated copies of the same chromosomes.
   d. sex chromosomes but not autosomes.
4. Crossing over involves each of the following with the exception of
   a. the transfer of DNA between two nonsister chromatids.
   b. the transfer of DNA between two sister chromatids.
   c. the formation of a synaptonemal complex.
   d. the alignment of homologous chromosomes.
5. During anaphase I
   a. sister chromatids separate and move to the poles.
   b. homologous chromosomes move to opposite poles.
   c. homologous chromosomes align at the middle of the cell.
   d. all the chromosomes align independently at the middle of the cell.
6. At metaphase I the kinetochores of sister chromatids are
   a. attached to microtubules from the same pole.
   b. attached to microtubules from opposite poles.
   c. held together with cohesin proteins.
   d. not attached to any microtubules.
7. What occurs during anaphase of meiosis II?
   a. The homologous chromosomes align.
   b. Sister chromatids are pulled to opposite poles.
   c. Homologous chromosomes are pulled to opposite poles.
   d. The haploid chromosomes line up.

APPLY

1. Which of the following does *not* contribute to genetic diversity?
   a. Independent assortment
   b. Recombination
   c. Metaphase of meiosis II
   d. Metaphase of meiosis I

2. How does DNA replication differ between mitosis and meiosis?
   a. DNA replication takes less time in meiosis because the cells are haploid.
   b. During meiosis, there is only one round of replication for two divisions.
   c. During mitosis, there is only one round of replication every other division.
   d. DNA replication is exactly the same in mitosis and meiosis.
3. Which of the following is *not* a distinct feature of meiosis?
   a. Pairing and exchange of genetic material between homologous chromosomes
   b. Attachment of sister kinetochores to spindle microtubules
   c. Movement of sister chromatids to the same pole
   d. Suppression of DNA replication
4. Which phase of meiosis I is most similar to the comparable phase in mitosis?
   a. Prophase I
   b. Metaphase I
   c. Anaphase I
   d. Telophase I
5. Structurally, meiotic cohesins have different components than mitotic cohesins. This leads to the following functional difference:
   a. During metaphase I, the sister kinetochores become attached to the same pole.
   b. Centromeres remain attached during anaphase I of meiosis.
   c. Centromeres remain attached through both divisions.
   d. Centromeric cohesins are destroyed at anaphase I, and cohesins along the arms are destroyed at anaphase II.
6. Mutations that affect DNA repair often also affect the accuracy of meiosis. This is because
   a. the proteins involved in the repair of double-strand breaks are also involved in crossing over.
   b. the proteins involved in DNA repair are also involved in sister chromatid cohesion.
   c. DNA repair only occurs on condensed chromosomes such as those found in meiosis.
   d. cohesin proteins are also necessary for DNA repair.

SYNTHESIZE

1. Diagram the process of meiosis for an imaginary cell with six chromosomes in a diploid cell.
   a. How many homologous pairs are present in this cell? Create a drawing that distinguishes between homologous pairs.
   b. Label each homologue to indicate whether it is maternal (M) or paternal (P).
   c. Draw a new cell showing how these chromosomes would arrange themselves during metaphase of meiosis I. Do all the maternal homologues have to line up on the same side of the cell?
   d. How would this picture differ if you were diagramming anaphase of meiosis II?
2. Mules are the offspring of the mating of a horse and a donkey. Mules are unable to reproduce. A horse has a total of 64 chromosomes, whereas donkeys have 62 chromosomes. Use your knowledge of meiosis to predict the diploid chromosome number of a mule. Propose a possible explanation for the inability of mules to reproduce.
3. Compare the processes of *independent assortment* and *crossing over*. Which process has the greatest influence on genetic diversity?
4. Aneuploid gametes are cells that contain the wrong number of chromosomes. Aneuploidy occurs as a result of *nondisjunction*, or lack of separation of the chromosomes during either phase of meiosis.
   a. At what point in meiotic cell division would nondisjunction occur?
   b. Imagine a cell had a diploid chromosome number of four. Create a diagram to illustrate the effects of nondisjunction of one pair of homologous chromosomes in meiosis I versus meiosis II.

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Chapter 12

Patterns of Inheritance

Chapter Outline

Introduction

Every living creature is a product of the long evolutionary history of life on Earth. All organisms share this history, but as far as we know, only humans wonder about the processes that led to their origin and investigate the possibilities. We are far from understanding everything about our origins, but we have learned a great deal. Like a partially completed jigsaw puzzle, the boundaries of this elaborate question have fallen into place, and much of the internal structure is becoming apparent. In this chapter, we discuss one piece of the puzzle—the enigma of heredity. Why do individuals, like the children in this picture, differ so much in appearance despite the fact that we are all members of the same species? And, why do members of a single family tend to resemble one another more than they resemble members of other families?

12.1 The Mystery of Heredity

Learning Outcomes

1. *Describe explanations for inheritance prior to Mendel.*
2. *Explain the advantages of Mendel's experimental system.*

As far back as written records go, patterns of resemblance among the members of particular families have been noted and commented on (figure 12.1), but there was no coherent model to explain these patterns. Before the 20th century, two concepts provided the basis for most thinking about heredity. The first was that heredity occurs within species. The second was that traits are transmitted directly from parents to offspring. Taken together, these ideas led to a view of inheritance as resulting from a blending of traits within fixed, unchanging species.

Figure 12.1 Heredity and family resemblance. Family resemblances are often strong—a visual manifestation of the mechanism of heredity.

Inheritance itself was viewed as traits being borne through fluid, usually identified as blood, that led to their blending in offspring. This older idea persists today in the use of the term "bloodlines" when referring to the breeding of domestic animals such as horses.

Taken together, however, these two classical assumptions led to a paradox. If no variation enters a species from outside, and if the variation within each species blends in every generation, then all members of a species should soon have the same appearance. It is clear that this does not happen—individuals within most species differ from one another, and they differ in characteristics that are transmitted from generation to generation.

Early plant biologists produced hybrids and saw puzzling results

The first investigator to achieve and document successful experimental **hybridizations** was Josef Kölreuter, who in 1760 cross-fertilized (or crossed, for short) different strains of tobacco and obtained fertile offspring. The hybrids differed in appearance from both parent strains. When individuals within the hybrid generation were crossed, their offspring were highly variable. Some of these offspring resembled plants of the hybrid generation (their parents), but a few resembled the original strains (their grandparents). The variation observed in second-generation offspring contradicts the theory of direct transmission. This can be seen as the beginning of modern genetics.

Over the next hundred years, other investigators elaborated on Kölreuter's work. T. A. Knight, an English landholder, in 1823 crossed two varieties of the garden pea, *Pisum sativum* (figure 12.2). One of these varieties had green seeds, and the other had yellow seeds. Both varieties were **true-breeding,** meaning that the offspring produced from self-fertilization remained uniform from one generation to the next. All of the progeny (offspring) of the cross between the two varieties had yellow seeds. Among the offspring of these hybrids, however, some plants produced yellow seeds and others, less common, produced green seeds.

Other investigators made observations similar to Knight's, namely that alternative forms of observed traits were being distributed among the offspring. A modern geneticist would say the alternative forms of each trait were **segregating** among the progeny of a mating, meaning that some offspring exhibited one form of a trait (yellow seeds), and other offspring from the same mating exhibited a different form (green seeds). This segregation of alternative forms of a trait provided the clue that led Gregor Mendel to his understanding of the nature of heredity.

Within these deceptively simple results were the makings of a scientific revolution. Nevertheless, another century passed before the process of segregation was fully appreciated.

Mendel used mathematics to analyze his crosses

Born in 1822 to peasant parents, Gregor Mendel (figure 12.3) was educated in a monastery and went on to study science and mathematics at the University of Vienna, where he failed his examinations for a teaching certificate. He returned to the monastery and spent the rest of his life there, eventually becoming abbot. In the garden of the monastery, Mendel initiated his own series of experiments on plant hybridization. The results of these experiments would ultimately change our views of heredity irrevocably.

Practical considerations for use of the garden pea

For his experiments, Mendel chose the garden pea, the same plant Knight and others had studied. The choice was a good one for several reasons. First, many earlier investigators had produced hybrid peas by crossing different varieties, so Mendel knew that he could expect to observe segregation of traits among the offspring.

Figure 12.2 The garden pea, *Pisum sativum*. Easy to cultivate and able to produce many distinctive varieties, the garden pea was a popular experimental subject in investigations of heredity as long as a century before Gregor Mendel's experiments.

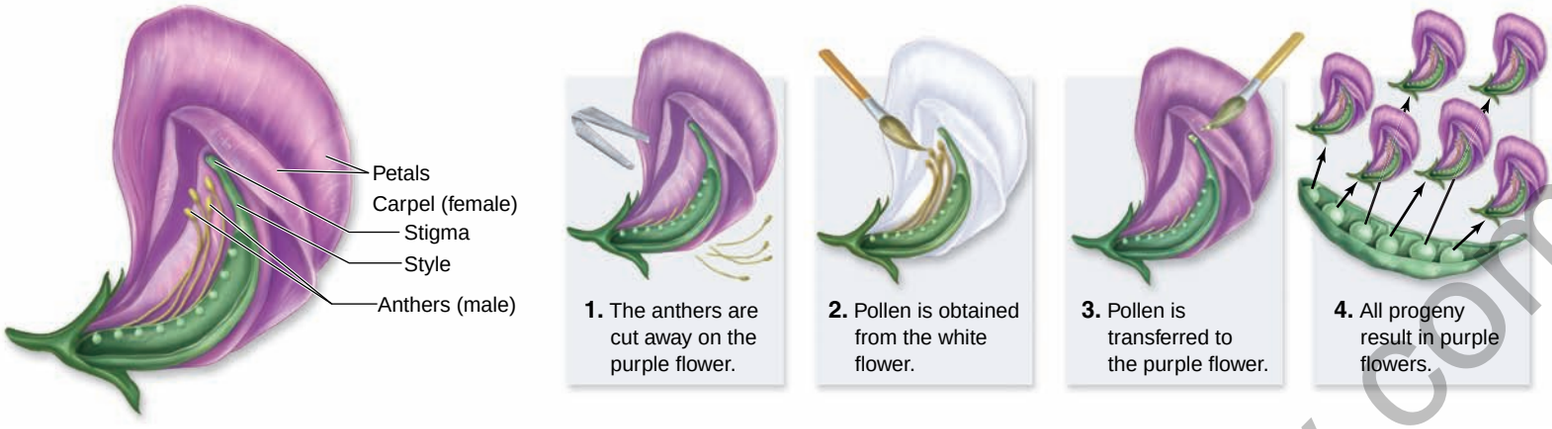


Figure 12.3 How Mendel conducted his experiments. In a pea plant flower, petals enclose both the male anther (containing pollen grains, which give rise to haploid sperm) and the female carpel (containing ovules, which give rise to haploid eggs). This ensures self-fertilization will take place unless the flower is disturbed. Mendel collected pollen from the anthers of a white flower, then placed that pollen onto the stigma of a purple flower with anthers removed. This cross fertilization yields all hybrid seeds that give rise to purple flowers. Using pollen from a white flower to fertilize a purple flower gives the same result.

Inquiry question

What confounding problems could have been seen if Mendel had chosen another plant with exposed male and female structures?

Second, a large number of pure varieties of peas were available. Mendel initially examined 34 varieties. Then, for further study, he selected lines that differed with respect to seven easily distinguishable traits, such as round versus wrinkled seeds and yellow versus green seeds, the latter a trait that Knight had studied.

Third, pea plants are small and easy to grow, and they have a relatively short generation time. A researcher can therefore conduct experiments involving numerous plants, grow several generations in a single year, and obtain results relatively quickly.

A fourth advantage of studying peas is that both the male and female sexual organs are enclosed within each pea flower (see figure 12.3), and gametes produced by the male and female parts of the same flower can fuse to form viable offspring, a process termed **self-fertilization.** This self-fertilization takes place automatically within an individual flower if it is not disturbed. It is also possible to prevent self-fertilization by removing a flower's male parts before fertilization occurs, then introduce pollen from a different strain, thus performing *cross-pollination* that results in *cross-fertilization* (see figure 12.3).

Mendel's experimental design

Mendel was careful to focus on only a few specific differences between the plants he was using and to ignore the countless other differences he must have seen. He also had the insight to realize that the differences he selected must be comparable. For example, he recognized that trying to study the inheritance of round seeds versus tall height would be useless.

Mendel usually conducted his experiments in three stages:

1. Mendel allowed plants of a given variety to self-cross for multiple generations to assure himself that the traits he was studying were indeed true-breeding, that is, transmitted unchanged from generation to generation.
2. Mendel then performed crosses between true-breeding varieties exhibiting alternative forms of traits. He also performed **reciprocal crosses:** using pollen from a white-flowered plant to fertilize a purple-flowered plant, then using pollen from a purple-flowered plant to fertilize a white-flowered plant.
3. Finally, Mendel permitted the hybrid offspring produced by these crosses to self-fertilize for several generations, allowing him to observe the inheritance of alternative forms of a trait. Most important, he counted the numbers of offspring exhibiting each trait in each succeeding generation.

This quantification of results is what distinguished Mendel's research from that of earlier investigators, who only noted differences in a qualitative way. Mendel's mathematical analysis of experimental results led to the inheritance model that we still use today.

Learning Outcomes Review 12.1

Prior to Mendel, concepts of inheritance did not form a consistent model. The dominant view was of blending inheritance, in which traits of parents were carried by fluid and "blended" in offspring. Plant hybridizers before Mendel, however, had already cast doubt on this model by observing characteristics in hybrids that seemed to change in second-generation offspring. Mendel's experiments with plants involved quantifying types of offspring and mathematically analyzing his observations.

- ***Which was more important to Mendel's success: his approach, or his choice of experimental material?***

12.2 Monohybrid Crosses: The Principle of Segregation

Learning Outcomes

1. *Evaluate the outcome of a monohybrid cross.*
2. *Explain Mendel's principle of segregation.*
3. *Compare the segregation of alleles with the behavior of homologues in meiosis.*

A *monohybrid cross* is a cross that follows only two variations on a single trait, such as white- and purple-colored flowers. This deceptively simple kind of cross can lead to important conclusions about the nature of inheritance.

The seven characteristics, or characters, Mendel studied in his experiments possessed two variants that differed from one another in ways that were easy to recognize and score (figure 12.4). We examine in detail Mendel's crosses with flower color. His experiments with other characters were similar, and they produced similar results.

The F_1 generation exhibits only one of two traits, without blending

When Mendel crossed white-flowered and purple-flowered plants, the hybrid offspring he obtained did not have flowers of intermediate color, as the hypothesis of blending inheritance would predict. Instead, in every case the flower color of the offspring resembled that of one of their parents. These offspring are customarily referred to as the **first filial generation,** or **F_1.** In a cross of white-flowered and purple-flowered plants, the F_1 offspring all had purple flowers, as other scientists had reported before Mendel.

Mendel referred to the form of each trait expressed in the F_1 plants as **dominant,** and to the alternative form that was not expressed in the F_1 plants as **recessive.** For each of the seven pairs of contrasting traits that Mendel examined, one of the pair proved to be dominant and the other recessive.

The F_2 generation exhibits both traits in a 3:1 ratio

After allowing individual F_1 plants to mature and self-fertilize, Mendel collected and planted the seeds from each plant to see what the offspring in the **second filial generation,** or **F_2,** would look like. He found that although most F_2 plants had purple flowers, some exhibited white flowers, the recessive trait. Although hidden in the F_1 generation, the recessive trait had reappeared among some F_2 individuals.

Believing the proportions of the F_2 types would provide some clue about the mechanism of heredity, Mendel counted the numbers of each type among the F_2 progeny. In the cross between the purple-flowered F_1 plants, he obtained a total of 929 F_2 individuals. Of these, 705 (75.9%) had purple flowers, and 224 (24.1%) had white flowers (see figure 12.4).

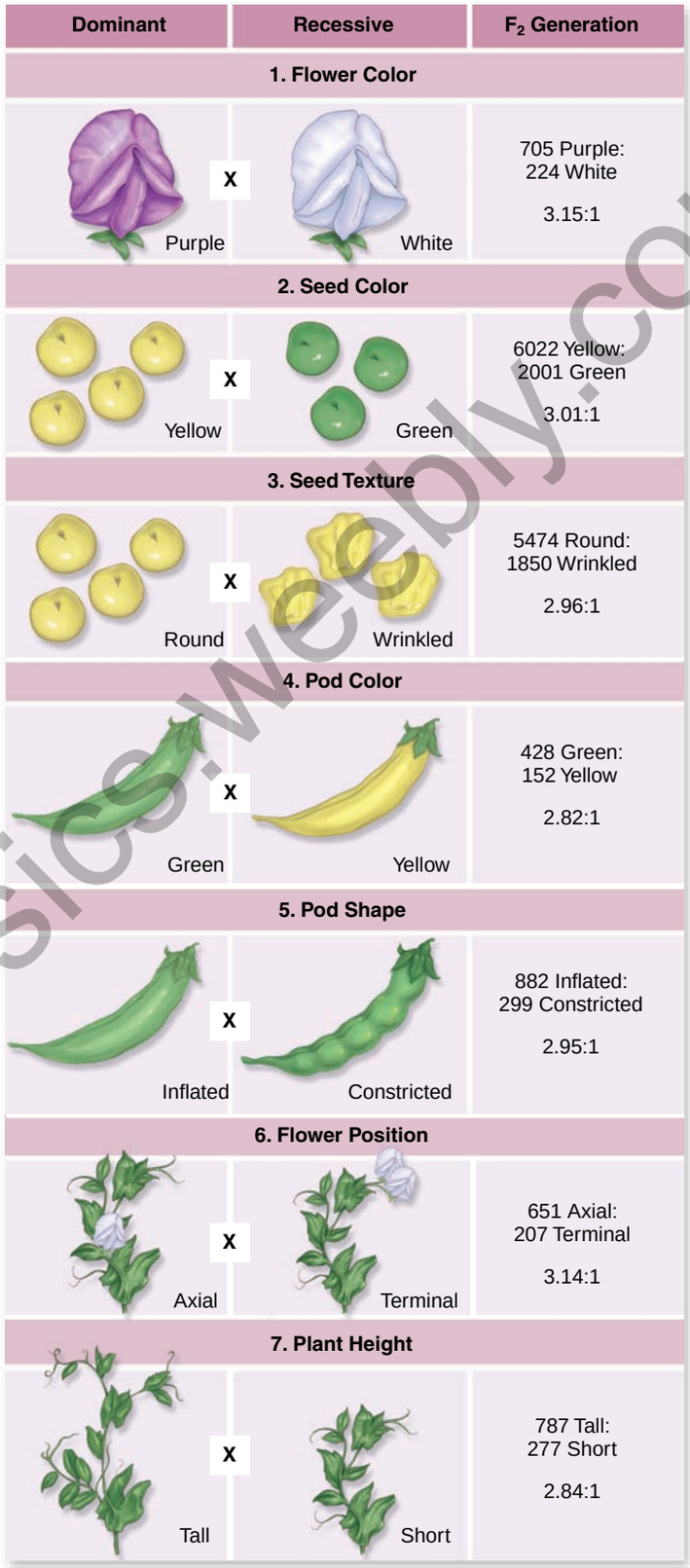


Figure 12.4 Mendel's seven traits. Mendel studied how differences among varieties of peas were inherited when the varieties were crossed. Similar experiments had been done before, but Mendel was the first to quantify the results and appreciate their significance. Results are shown for seven different monohybrid crosses. The F_1 generation is not shown in the table.

Approximately ¼ of the F_2 individuals, therefore, exhibited the recessive form of the character.

Mendel obtained the same numerical result with the other six characters he examined: Of the F_2 individuals, ¾ exhibited the dominant trait, and ¼ displayed the recessive trait (see figure 12.4). In other words, the dominant-to-recessive ratio among the F_2 plants was always close to 3:1.

The 3:1 ratio is actually 1:2:1

Mendel went on to examine how the F_2 plants passed traits to subsequent generations. He found that plants exhibiting the recessive trait were always true-breeding. For example, the white-flowered F_2 individuals reliably produced white-flowered offspring when they were allowed to self-fertilize. By contrast, only ⅓ of the dominant, purple-flowered F_2 individuals (¼ of all F_2 offspring) proved true-breeding, but ⅔ were not. This last class of plants produced dominant and recessive individuals in the third filial generation (F_3) in a 3:1 ratio.

This result suggested that, for the entire sample, the 3:1 ratio that Mendel observed in the F_2 generation was really a disguised 1:2:1 ratio: ¼ true-breeding dominant individuals, ½ not-true-breeding dominant individuals, and ¼ true-breeding recessive individuals (figure 12.5).

Mendel's Principle of Segregation explains monohybrid observations

From his experiments, Mendel was able to understand four things about the nature of heredity:

- The plants he crossed did not produce progeny of intermediate appearance, as a hypothesis of blending inheritance would have predicted. Instead, different plants inherited each trait intact, as a discrete characteristic.
- For each pair of alternative forms of a trait, one alternative was not expressed in the F_1 hybrids, although it reappeared in some F_2 individuals. *The trait that "disappeared" must therefore be latent (present but not expressed) in the F_1 individuals.*
- The pairs of alternative traits examined were segregated among the progeny of a particular cross, some individuals exhibiting one trait and some the other.
- These alternative traits were expressed in the F_2 generation in the ratio of ¾ dominant to ¼ recessive. This characteristic 3:1 segregation is referred to as the **Mendelian ratio** for a monohybrid cross.

Mendel's five-element model

To explain these results, Mendel proposed a simple model that has become one of the most famous in the history of science, containing simple assumptions and making clear predictions. The model has five elements:

1. Parents do not transmit physiological traits directly to their offspring. Rather, they transmit discrete information for the traits, what Mendel called "factors." We now call these factors *genes*.

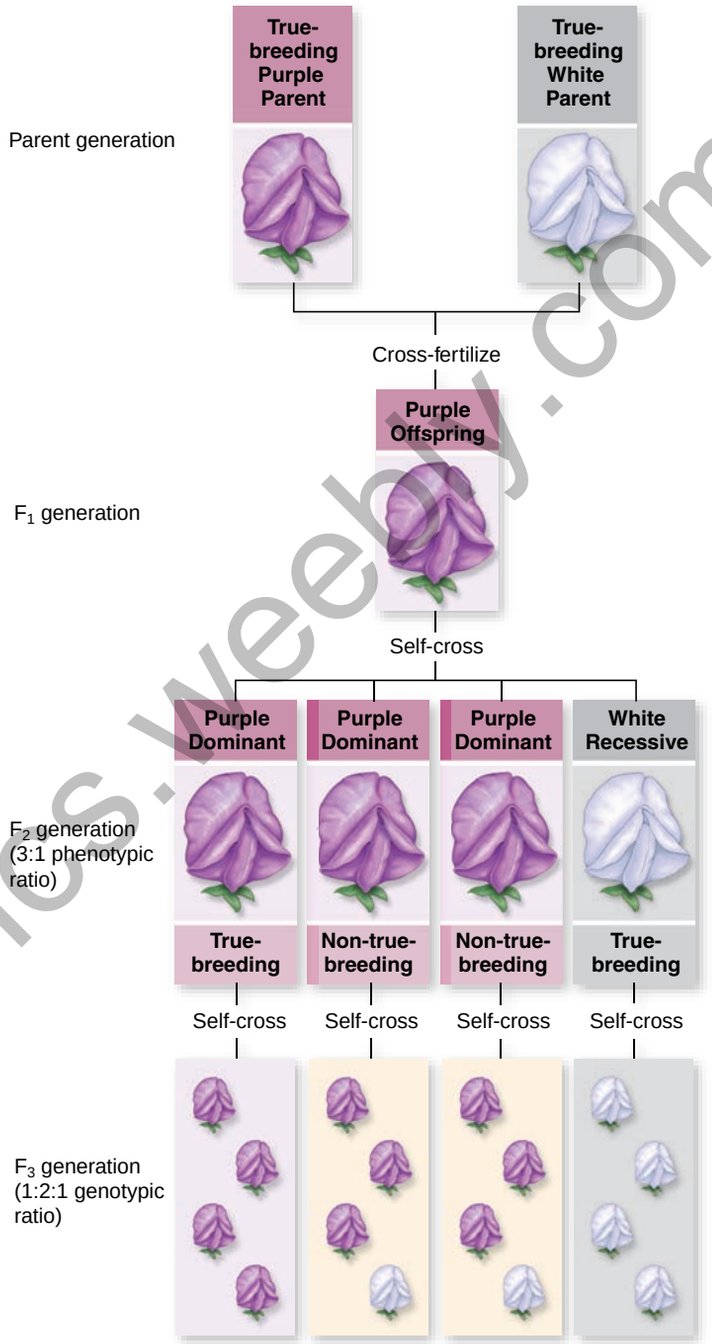


Figure 12.5 The F_2 generation is a disguised 1:2:1 ratio. By allowing the F_2 generation to self-fertilize, Mendel found from the offspring (F_3) that the ratio of F_2 plants was 1 true-breeding dominant: 2 not-true-breeding dominant: and 1 true-breeding recessive.

2. Each individual receives one copy of each gene from each parent. We now know that genes are carried on chromosomes, and each adult individual is diploid, with one set of chromosomes from each parent.
3. Not all copies of a gene are identical. The alternative forms of a gene are called **alleles.** When two haploid gametes containing the same allele fuse during fertilization, the resulting offspring is said to be **homozygous.** When the two haploid gametes contain different alleles, the resulting offspring is said to be **heterozygous.**

4. The two alleles remain discrete—they neither blend with nor alter each other. Therefore, when the individual matures and produces its own gametes, the alleles segregate randomly into these gametes.
5. The presence of a particular allele does not ensure that the trait it encodes will be expressed. In heterozygous individuals, only one allele is expressed (the dominant one), and the other allele is present but unexpressed (the recessive one).

Geneticists now refer to the total set of alleles that an individual contains as the individual's **genotype.** The physical appearance or other observable characteristics of that individual, which result from an allele's expression, is termed the individual's **phenotype.** In other words, the genotype is the blueprint, and the phenotype is the visible outcome in an individual.

This also allows us to present Mendel's ratios in more modern terms. The 3:1 ratio of dominant to recessive is the monohybrid phenotypic ratio. The 1:2:1 ratio of homozygous dominant to heterozygous to homozygous recessive is the monohybrid genotypic ratio. The genotypic ratio "collapses" into the phenotypic ratio due to the action of the dominant allele making the heterozygote appear the same as homozygous dominant.

The principle of segregation

Mendel's model accounts for the ratios he observed in a neat and satisfying way. His main conclusion—that alternative alleles for a character segregate from each other during gamete formation and remain distinct—has since been verified in many other organisms. It is commonly referred to as Mendel's first law of heredity, or the **Principle of Segregation.** It can be simply stated as: *The two alleles for a gene segregate during gamete formation and are rejoined at random, one from each parent, during fertilization.*

The physical basis for allele segregation is the behavior of chromosomes during meiosis. As you saw in chapter 11, homologues for each chromosome disjoin during anaphase I of meiosis. The second meiotic division then produces gametes that contain only one homologue for each chromosome.

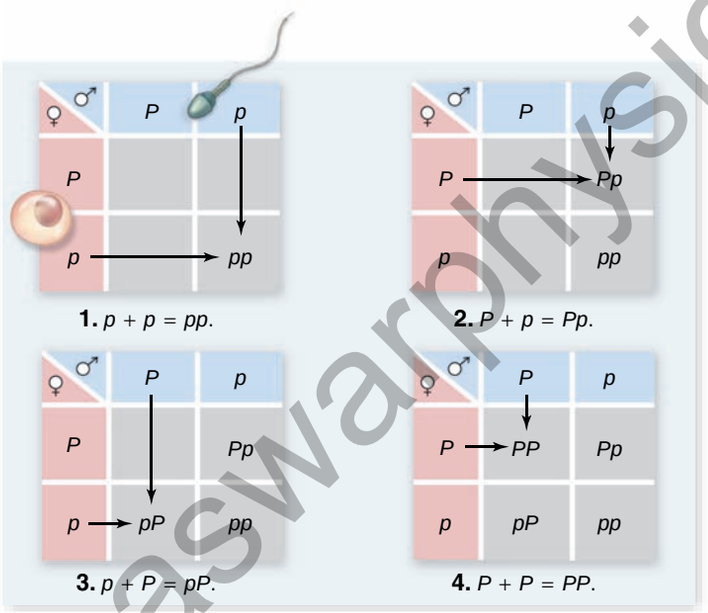
It is a tribute to Mendel's intellect that his analysis arrived at the correct scheme, even though he had no knowledge of the cellular mechanisms of inheritance; neither chromosomes nor meiosis had yet been described.

The Punnett square allows symbolic analysis

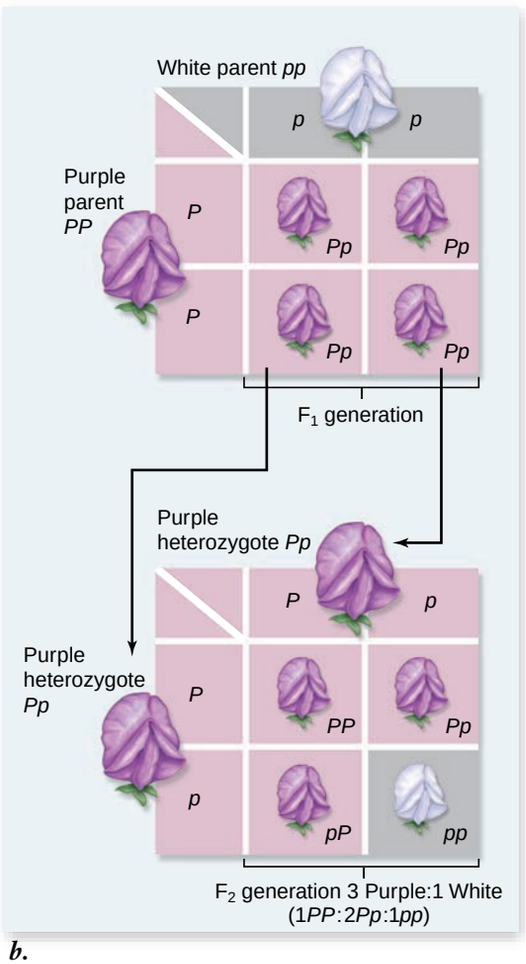
To test his model, Mendel first expressed it in terms of a simple set of symbols. He then used the symbols to interpret his results.

Consider again Mendel's cross of purple-flowered with white-flowered plants. By convention, we assign the symbol *P* (uppercase) to the dominant allele, associated with the production of purple flowers, and the symbol *p* (lowercase) to the recessive allele, associated with the production of white flowers.

In this system, the genotype of an individual that is true-breeding for the recessive white-flowered trait would be designated *pp*. Similarly, the genotype of a true-breeding purple-flowered



a.



b.

Figure 12.6 Using a Punnett square to analyze Mendel's cross.
a. To make a Punnett square, place the different female gametes along the side of a square and the different male gametes along the top. Each potential zygote is represented as the intersection of a vertical line and a horizontal line. ***b.*** In Mendel's cross of purple by white flowers, each parent makes only one type of gamete. The F_1 are all purple, *Pp*, heterozygotes. These F_1 offspring make two types of gametes that can be combined to produce three kinds of F_2 offspring: *PP* homozygous dominant (purple); *Pp* heterozygous (also purple); and *pp* homozygous recessive (white). The phenotypic ratio is 3 purple:1 white. The genotypic ratio is 1 *PP*:2 *Pp*:1 *pp*.

TABLE 12.1 Some Dominant and Recessive Traits in Humans

Recessive Traits	Phenotypes	Dominant Traits	Phenotypes
Albinism	Lack of melanin pigmentation	Middigital hair	Presence of hair on middle segment of fingers
Alkaptonuria	Inability to metabolize homogentisic acid	Brachydactyly	Short fingers
Red-green color blindness	Inability to distinguish red or green wavelengths of light	Huntington disease	Degeneration of nervous system, starting in middle age
Cystic fibrosis	Abnormal gland secretion, leading to liver degeneration and lung failure	Phenylthiocarbamide (PTC) sensitivity	Ability to taste PTC as bitter
Duchenne muscular dystrophy	Wasting away of muscles during childhood	Camptodactyly	Inability to straighten the little finger
Hemophilia	Inability of blood to clot properly, some clots form but the process is delayed	Hypercholesterolemia (the most common human Mendelian disorder)	Elevated levels of blood cholesterol and risk of heart attack
Sickle cell anemia	Defective hemoglobin that causes red blood cells to curve and stick together	Polydactyly	Extra fingers and toes

individual would be designated *PP*. In contrast, a heterozygote would be designated *Pp* (dominant allele first). Using these conventions and denoting a cross between two strains with ×, we can symbolize Mendel's original purple × white cross as *PP* × *pp*.

Because a white-flowered parent (*pp*) can produce only *p* gametes, and a true-breeding purple-flowered parent (*PP*, *homozygous dominant*) can produce only *P* gametes, the union of these gametes can produce only heterozygous *Pp* offspring in the F_1 generation. Because the *P* allele is dominant, all of these F_1 individuals are expected to have purple flowers.

When F_1 individuals are allowed to self-fertilize, the *P* and *p* alleles segregate during gamete formation to produce both *P* gametes and *p* gametes. Their subsequent union at fertilization to form F_2 individuals is random.

The F_2 possibilities may be visualized in a simple diagram called a **Punnett square,** named after its originator, the English geneticist R. C. Punnett (figure 12.6*a*). Mendel's model, analyzed in terms of a Punnett square, clearly predicts that the F_2 generation should consist of ¾ purple-flowered plants and ¼ white-flowered plants, a phenotypic ratio of 3:1 (figure 12.6*b*).

Some human traits exhibit dominant/recessive inheritance

A number of human traits have been shown to display both dominant and recessive inheritance (table 12.1 provides a sample of these). Researchers cannot perform controlled crosses in humans the way Mendel did with pea plants, instead geneticists study crosses that have already been performed—in other words, family histories. The organized methodology we use is a **pedigree,** a consistent graphical representation of matings and offspring over multiple generations for a particular trait. The information in the pedigree may allow geneticists to deduce a model for the mode of inheritance of the trait. In analyzing these pedigrees, it is important to realize that disease-causing alleles are usually quite rare in the general population.

A dominant pedigree: Juvenile glaucoma

One of the most extensive pedigrees yet produced traced the inheritance of a form of blindness caused by a dominant allele. The disease allele causes a form of hereditary juvenile glaucoma. The disease causes degeneration of nerve fibers in the optic nerve, leading to blindness.

This pedigree followed inheritance over three centuries, following the origin back to a couple in a small town in northwestern France who died in 1495. A small portion of this pedigree is shown in figure 12.7. The dominant nature of the trait is obvious from the

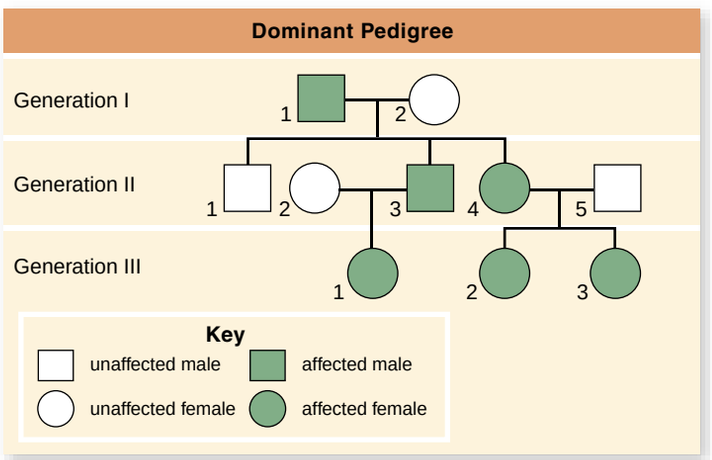


Figure 12.7 Dominant pedigree for hereditary juvenile glaucoma. Males are shown as squares and females are shown as circles. Affected individuals are shown shaded. The dominant nature of this trait can be seen in the trait appearing in every generation, a feature of dominant traits.

Inquiry question

If one of the affected females in the third generation married an unaffected male, could she produce unaffected offspring? If so, what are the chances of having unaffected offspring?

fact that every generation shows the trait. This is extremely unlikely for a recessive trait as it would require large numbers of unrelated individuals to be carrying the disease allele.

A recessive pedigree: Albinism

An example of inheritance of a recessive human trait is albinism, a condition in which the pigment melanin is not produced. Long thought to be due to a single gene, multiple genes are now known that lead to albinism; the common feature is the loss of pigment from hair, skin, and eyes. The loss of pigment makes albinistic individuals sensitive to the sun. The tanning effect we are all familiar with from exposure to the sun is due to increased numbers of pigment-producing cells, and increased production of pigment. This is lacking in albinistic individuals due to the lack of any pigment to begin with.

The pedigree in figure 12.8 is for a form of albinism due to a nonfunctional allele of the enzyme tyrosinase, which is required for the formation of melanin pigment. The genetic characteristics of this form of albinism are: females and males are affected equally, most affected individuals have unaffected parents, a single affected parent usually does not have affected offspring, and affected offspring are more frequent when parents are related. Each of these features can be see in figure 12.8, and all of this fits a recessive mode of inheritance.

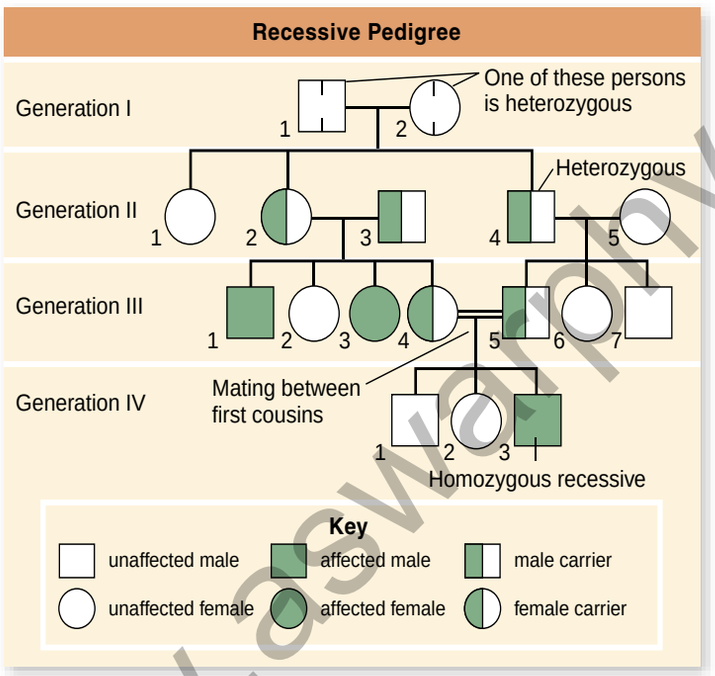


Figure 12.8 Recessive pedigree for albinism. One of the two individuals in the first generation must be heterozygous and individuals II-2 and II-4 must be heterozygous. Notice that for each affected individual, neither parent is affected, but both must be heterozygous (carriers). The double line indicates a consanguineous mating (between relatives) that, in this case, produced affected offspring.

Inquiry question

From a genetic disease standpoint, why is it never advisable for close relatives to mate and have children?

Learning Outcomes Review 12.2

Mendel's monohybrid crosses refute the idea of blending. One trait disappears in the first generation (F_1), then reappears in a predictable ratio in the next (F_2). The trait observable in the F_1 is called dominant, and the other recessive. In the F_2, the ratio of observed dominant offspring to recessive is 3:1, and this represents a ratio of 1 homozygous dominant to 2 heterozygous to 1 homozygous recessive. The Principle of Segregation states that alleles segregate into different gametes, which randomly combine at fertilization. The physical basis for segregation is the separation of homologues during anaphase I of meiosis.

- ***What fraction of tall F_2 plants are true-breeding?***

12.3 Dihybrid Crosses: The Principle of Independent Assortment

Learning Outcomes

1. ***Evaluate the outcome of a dihybrid cross.***
2. ***Explain Mendel's Principle of Independent Assortment.***
3. ***Understand the physical basis of independent assortment.***

The Principle of Segregation explains the behavior of alternative forms of a single trait in a monohybrid cross. The next step is to extend this to follow the behavior of two different traits in a single cross: a **dihybrid cross.**

With an understanding of the behavior of single traits, Mendel went on to ask if different traits behaved independently in hybrids. He first established a series of true-breeding lines of peas that differed in two of the seven characters he had studied. He then crossed contrasting pairs of the true-breeding lines to create heterozygotes. These heterozygotes are now doubly heterozygous, or dihybrid. Finally, he self-crossed the dihybrid F_1 plants to produce an F_2 generation, and counted all progeny types.

Traits in a dihybrid cross behave independently

Consider a cross involving different seed shape alleles (round, *R*, and wrinkled, *r*) and different seed color alleles (yellow, *Y*, and green, *y*). Crossing round yellow (*RR YY*) with wrinkled green (*rr yy*), produces heterozygous F_1 individuals having the same phenotype (namely round and yellow) and the same genotype (*Rr Yy*). Allowing these dihybrid F_1 individuals to self-fertilize produces an F_2 generation.

The F_2 generation exhibits four types of progeny in a 9:3:3:1 ratio

In analyzing these results, we first consider the number of possible phenotypes. We expect to see the two parental phenotypes: round yellow and wrinkled green. If the traits behave independently, then we can also expect one trait from each parent to produce plants with round green seeds and others with wrinkled yellow seeds.

Next consider what types of gametes the F_1 individuals can produce. Again, we expect the two types of gametes found in the parents: *RY* and *ry*. If the traits behave independently, then we can also expect the gametes *Ry* and *rY*. Using modern language, two genes each with two alleles can be combined four ways to produce these gametes: *RY*, *ry*, *Ry*, and *rY*.

A dihybrid Punnett square

We can then construct a Punnett square with these gametes to generate all possible progeny. This is a 4 × 4 square with 16 possible outcomes. Filling in the Punnett square produces all possible offspring (figure 12.9). From this we can see that there are 9 round yellow, 3 wrinkled yellow, 3 round green, and 1 wrinkled green. This predicts a phenotypic ratio of 9:3:3:1 for traits that behave independently.

Mendel's Principle of Independent Assortment explains dihybrid results

What did Mendel actually observe? From a total of 556 seeds from self-fertilized dihybrid plants, he observed the following results:

- 315 round yellow (signified *R__ Y__*, where the underscore indicates the presence of either allele),
- 108 round green *(R__ yy)*,
- 101 wrinkled yellow *(rr Y__)*, and
- 32 wrinkled green *(rr yy)*.

These results are very close to a 9:3:3:1 ratio. (The expected 9:3:3:1 ratio from this many offspring would be 313:104:104:35.)

The alleles of two genes appeared to behave independently of each other. Mendel referred to this phenomenon as the traits assorting independently. Note that this *independent assortment* of different genes in no way alters the segregation of individual pairs of alleles for each gene. Round versus wrinkled seeds occur in a ratio of approximately 3:1 (423:133); so do yellow versus green seeds (416:140). Mendel obtained similar results for other pairs of traits.

We call this Mendel's second law of heredity, or the **Principle of Independent Assortment.** This can also be stated simply: *In a dihybrid cross, the alleles of each gene assort independently*. A more precise statement would be: *the segregation of different allele pairs is independent*. This statement more closely ties independent assortment to the behavior of chromosomes during meiosis (see chapter 11). The independent alignment of different homologous chromosome pairs during metaphase I leads to the independent segregation of the different allele pairs.

Learning Outcomes Review 12.3

Mendel's analysis of dihybrid crosses revealed that the segregation of allele pairs for different traits is independent; this finding is known as Mendel's Principle of Independent Assortment. When individuals that differ in two traits are crossed, and their progeny are intercrossed, the result is four different types that occur in a ratio of 9:3:3:1, Mendel's dihybrid ratio. This occurs because of the independent behavior of different homologous pairs of chromosomes during meiosis I.

- ***Which is more important in terms of explaining Mendel's laws, meiosis I or meiosis II?***

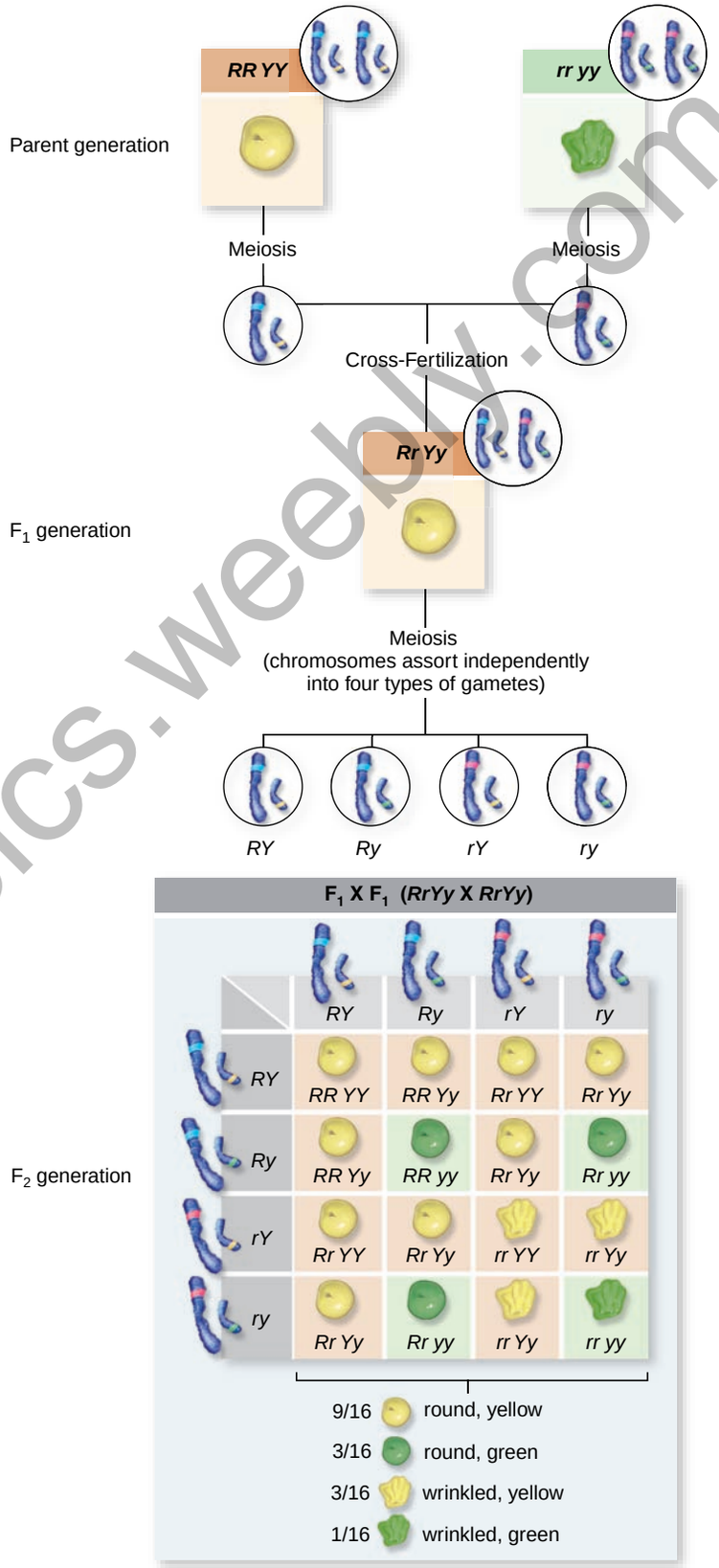


Figure 12.9 Analyzing a dihybrid cross. This Punnett square shows the results of Mendel's dihybrid cross between plants with round yellow seeds and plants with wrinkled green seeds. The ratio of the four possible combinations of phenotypes is predicted to be 9:3:3:1, the ratio that Mendel found.

12.4 Probability: Predicting the Results of Crosses

Learning Outcomes

1. *Understand the rule of addition and the rule of multiplication.*
2. *Apply the rules of probability to genetic crosses.*

Probability allows us to predict the likelihood of the outcome of random events. Because the behavior of different chromosomes during meiosis is independent, we can use probability to predict the outcome of crosses. The probability of an event that is certain to happen is equal to 1. In contrast, an event that can never happen has a probability of 0. Therefore, probabilities for all other events have fractional values, between 0 and 1. For instance, when you flip a coin, two outcomes are possible; there is only one way to get the event "heads" so the probability of heads is one divided by two, or ½. In the case of genetics, consider a pea plant heterozygous for the flower color alleles *P* and *p*. This individual can produce two types of gametes in equal numbers, again due to the behavior of chromosomes during meiosis. There is one way to get a *P* gamete, so the probability of any particular gamete carrying a *P* allele is 1 divided by 2 or ½, just like the coin toss.

Two probability rules help predict monohybrid cross results

We can use probability to make predictions about the outcome of genetic crosses using only two simple rules. Before we describe these rules and their uses, we need another definition. We say that two events are *mutually exclusive* if both cannot happen at the same time. The heads and tails of a coin flip are examples of mutually exclusive events. Notice that this is different from two consecutive coin flips where you can get two heads or two tails. In this case, each coin flip represents an *independent event*. It is the distinction between independent and mutually exclusive events that forms the basis for our two rules.

The rule of addition

Consider a six-sided die instead of a coin: for any roll of the die, only one outcome is possible, and each of the possible outcomes are mutually exclusive. The probability of any particular number coming up is ⅙. The probability of either of two different numbers is the sum of the individual probabilities, or restated as the **rule of addition:**

For two mutually exclusive events, the probability of either event occurring is the sum of the individual probabilities.

Probability of rolling either a 2 or a 6
is = $^1/_6 + {}^1/_6 = {}^2/_6 = {}^1/_3$

To apply this to our cross of heterozygous purple F_1, four mutually exclusive outcomes are possible: *PP*, *Pp*, *pP*, and *pp*. The probability of being heterozygous is the same as the probability of being either *Pp* or *pP*, or ¼ plus ¼, or ½.

Probability of F_2 heterozygote = ¼*Pp* + ¼*pP* = ½

In the previous example, of 379 total offspring, we would expect about 190 to be heterozygotes. (The actual number is 189.5.)

The rule of multiplication

The second rule, and by far the most useful for genetics, deals with the outcome of independent events. This is called the **product rule,** or **rule of multiplication,** and it states that the probability of two independent events both occurring is the *product* of their individual probabilities.

We can apply this to a monohybrid cross in which offspring are formed by gametes from each of two parents. For any particular outcome then, this is due to two independent events: the formation of two different gametes. Consider the purple F_1 parents from earlier. They are all *Pp* (heterozygotes), so the probability that a particular F_2 individual will be *pp* (homozygous recessive) is the probability of receiving a *p* gamete from the male (½) times the probability of receiving a *p* gamete from the female (½), or ¼:

Probability of *pp* homozygote = ½*p* (male parent) × ½*p* (female parent) = ¼*pp*

This is actually the basis for the Punnett square that we used before. Each cell in the square was the product of the probabilities of the gametes that contribute to the cell. We then use the addition rule to sum the probabilities of the mutually exclusive events that make up each cell.

We can use the result of a probability calculation to predict the number of homozygous recessive offspring in a cross between heterozygotes. For example, out of 379 total offspring, we would expect about 95 to exhibit the homozygous recessive phenotype. (The actual calculated number is 94.75.)

Dihybrid cross probabilities are based on monohybrid cross probabilities

Probability analysis can be extended to the dihybrid case. For our purple F_1 by F_1 cross, there are four possible outcomes, three of which show the dominant phenotype. Thus the probability of any offspring showing the dominant phenotype is ¾, and the probability of any offspring showing the recessive phenotype is ¼. Now we can use this and the product rule to predict the outcome of a dihybrid cross. We will use our example of seed shape and color from earlier, but now examine it using probability.

If the alleles affecting seed shape and seed color segregate independently, then the probability that a particular pair of alleles for seed shape would occur together with a particular pair of alleles for seed color is the product of the individual probabilities for each pair. For example, the probability that an individual with wrinkled green seeds (*rr yy*) would appear in the F_2 generation would be equal to the probability of obtaining wrinkled seeds (¼) times the probability of obtaining green seeds (¼), or $^1/_{16}$.

Probability of *rr yy* = ¼ *rr* × ¼ *yy* = $^1/_{16}$ *rr yy*

Because of independent assortment, we can think of the dihybrid cross of consisting of two independent monohybrid crosses; since these are independent events, the product rule applies. So, we can calculate the probabilities for each dihybrid phenotype:

Probability of round yellow (*R*__ *Y*__) =
¾ *R*__ × ¾ *Y*__ = 9/16

Probability of round green (*R*__ *yy*) =
¾ *R*__ × ¼ *yy* = 3/16

Probability of wrinkled yellow (*rr Y*__) =
¼ *rr* × ¾ *Y*__ = 3/16

Probability of wrinkled green (*rr yy*) =
¼ *rr* ×¼ *yy* = 1/16

The hypothesis that color and shape genes are independently sorted thus predicts that the F_2 generation will display a 9:3:3:1 phenotypic ratio. These ratios can be applied to an observed total offspring to predict the expected number in each phenotypic group. The underlying logic and the results are the same as obtained using the Punnett square.

Learning Outcomes Review 12.4

The rule of addition states that the probability of either of two events occurring is the sum of their individual probabilities. The rule of multiplication states that the probability of two independent events both occurring is the product of their individual probabilities. These rules can be applied to genetic crosses to determine the probability of particular genotypes and phenotypes. Results can then be compared against these predictions.

- ***How would you calculate the probability of all dominant phenotype F_2 progeny in a trihybrid cross?***

12.5 The Testcross: Revealing Unknown Genotypes

Learning Outcome

1. ***Interpret data from test crosses to infer genotypes.***

To test his model further, Mendel devised a simple and powerful procedure called the **testcross.** In a testcross, an individual with unknown genotype is crossed with the homozygous recessive genotype—that is, the recessive parental variety. The contribution of the homozygous recessive parent can be ignored, because this parent can contribute only recessive alleles.

Consider a purple-flowered pea plant. It is impossible to tell whether such a plant is homozygous or heterozygous simply by looking at it. To learn its genotype, you can perform a testcross to a white-flowered plant. In this cross, the two possible test plant genotypes will give different results (figure 12.10):

Alternative 1: Unknown individual is homozygous dominant (*PP*) *PP* × *pp:* All offspring have purple flowers (*Pp*).

Alternative 2: Unknown individual is heterozygous (*Pp*) *Pp* × *pp:* ½ of offspring have white flowers (*pp*), and ½ have purple flowers (*Pp*).

Put simply, the appearance of the recessive phenotype in the offspring of a testcross indicates that the test individual's genotype is heterozygous.

For each pair of alleles Mendel investigated, he observed phenotypic F_2 ratios of 3:1 (see figure 12.4) and testcross ratios of 1:1, just as his model had predicted. Testcrosses can also be used to determine the genotype of an individual when two genes are involved. Mendel often

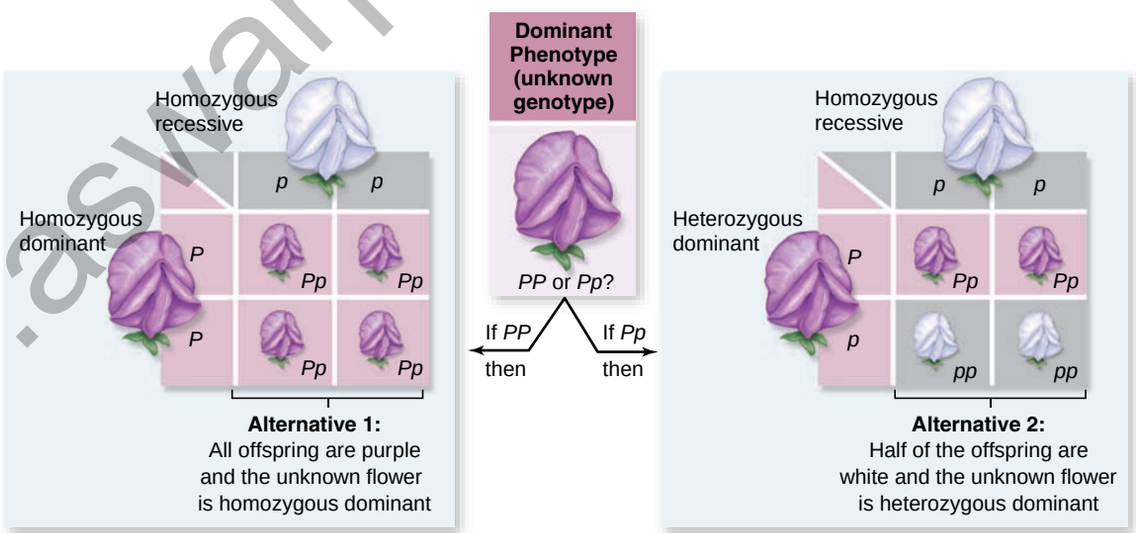


Figure 12.10 A testcross. To determine whether an individual exhibiting a dominant phenotype, such as purple flowers, is homozygous or heterozygous for the dominant allele, Mendel crossed the individual in question with a plant that he knew to be homozygous recessive—in this case, a plant with white flowers.

TABLE 12.2 Dihybrid Testcross

Actual Genotype	Results of Testcross	
	Trait A	Trait B
AABB	Trait A breeds true	Trait B breeds true
AaBB	———	Trait B breeds true
AABb	Trait A breeds true	———
AaBb	———	———

performed testcrosses to verify the genotypes of dominant-appearing F_2 individuals.

An F_2 individual exhibiting both dominant traits (*A__ B__*) might have any of the following genotypes: *AABB*, *AaBB*, *AABb*, or *AaBb*. By crossing dominant-appearing F_2 individuals with homozygous recessive individuals (that is, *A__ B__* × *aabb*), Mendel was able to determine whether either or both of the traits bred true among the progeny, and so to determine the genotype of the F_2 parent (table 12.2).

Testcrossing is a powerful tool that simplifies genetic analysis. We will use this method of analysis in the next chapter, when we explore genetic mapping.

Learning Outcome Review 12.5

Individuals showing the dominant phenotype can be either homozygous dominant or heterozygous. Unknown genotypes can be revealed using a testcross, which is a cross to a homozygous recessive individual. Heterozygotes produce both dominant and recessive phenotypes in equal numbers as a result of the testcross.

- ***In a dihybrid testcross of a doubly heterozygous individual, what would be the expected phenotypic ratio?***

12.6 *Extensions to Mendel*

Learning Outcomes

1. ***Describe how assumptions in Mendel's model result in oversimplification.***
2. ***Discuss a genetic explanation for continuous variation.***
3. ***Explain the genetic basis for observed alterations to Mendel's ratios.***

Although Mendel's results did not receive much notice during his lifetime, three different investigators independently rediscovered his pioneering paper in 1900, 16 years after his death. They came across it while searching the literature in preparation for publishing their own findings, which closely resembled those Mendel had presented more than 30 years earlier.

In the decades following the rediscovery of Mendel's ideas, many investigators set out to test them. However, scientists attempting to confirm Mendel's theory often had trouble obtaining the same simple ratios he had reported.

The reason that Mendel's simple ratios were not obtained had to do with the traits that others examined. A number of assumptions are built into Mendel's model that are oversimplifications. These assumptions include that each trait is specified by a single gene with two alternative alleles; that there are no environmental effects; and that gene products act independently. The idea of dominance also hides a wealth of biochemical complexity. In the following sections, you'll see how Mendel's simple ideas can be extended to provide a more complete view of genetics (table 12.3).

In polygenic inheritance, more than one gene can affect a single trait

Often, the relationship between genotype and phenotype is more complicated than a single allele producing a single trait. Most phenotypes also do not reflect simple two-state cases like purple or white flowers.

Consider Mendel's crosses between tall and short pea plants. In reality, the "tall" plants actually have normal height, and the "short" plants are dwarfed by an allele at a single gene. But in most species, including humans, height varies over a continuous range, rather than having discrete values. This continuous distribution of a phenotype has a simple genetic explanation: more than one gene is at work. The mode of inheritance operating in this case is often called **polygenic inheritance.**

In reality, few phenotypes result from the action of only one gene. Instead, most characters reflect multiple additive contributions to the phenotype by several genes. When multiple genes act jointly to influence a character, such as height or weight, the character often shows a range of small differences. When these genes segregate independently, a gradation in the degree of difference can be observed when a group consisting of many individuals is examined (figure 12.11). We call this gradation **continuous variation,** and we call such traits **quantitative traits.** The greater the number of genes influencing a character, the more continuous the expected distribution of the versions of that character.

This continuous variation in traits is similar to blending different colors of paint: Combining one part red with seven parts white, for example, produces a much lighter shade of pink than does combining five parts red with three parts white. Different ratios of red to white result in a continuum of shades, ranging from pure red to pure white.

Often, variations can be grouped into categories, such as different height ranges. Plotting the numbers in each height category produces a curve called a *histogram*, such as that shown in figure 12.11. The bell-shaped histogram approximates an idealized *normal distribution*, in which the central tendency is characterized by the mean, and the spread of the curve indicates the amount of variation.

Even simple-appearing traits can have this kind of polygenic basis. For example, human eye colors are often described in

TABLE 12.3 When Mendel's Laws/Results May Not Be Observed

Genetic Occurrence	Definition	Examples
Polygenic inheritance	More than one gene can affect a single trait.	• Four genes are involved in determining eye color. • Human height
Pleiotropy	A single gene can affect more than one trait.	• A pleiotropic allele dominant for yellow fur in mice is recessive for a lethal developmental defect. • Cystic fibrosis • Sickle cell anemia
Multiple alleles for one gene	Genes may have more than two alleles.	ABO blood types in humans
Dominance is not always complete	• In incomplete dominance the heterozygote is intermediate. • In codominance no single allele is dominant, and the heterozygote shows some aspect of both homozygotes.	• Japanese four o'clocks • Human blood groups
Environmental factors	Genes may be affected by the environment.	Siamese cats
Gene interaction	Products of genes can interact to alter genetic ratios.	• The production of a purple pigment in corn • Coat color in mammals

simple terms with brown dominant to blue, but this is actually incorrect. Extensive analysis indicates that at least four genes are involved in determining eye color. This leads to more complex inheritance patterns than initially reported. For example, blue-eyed parents can have brown-eyed offspring, although it is rare.

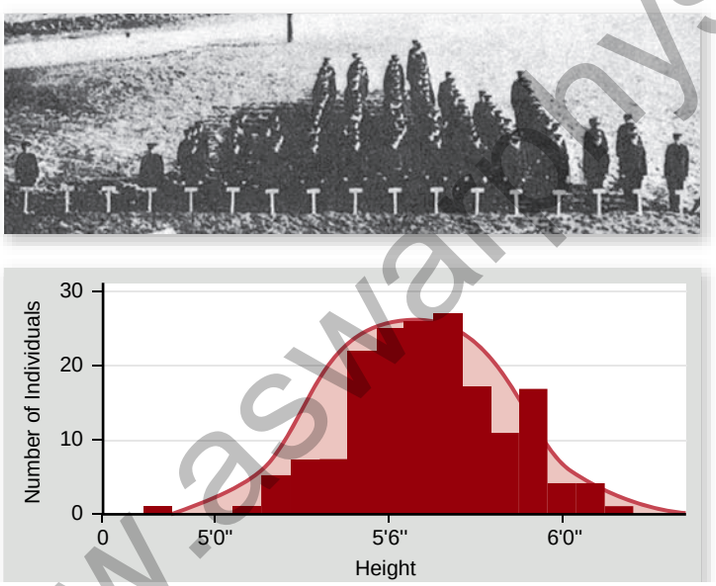


Figure 12.11 Height is a continuously varying trait. The photo and accompanying graph show variation in height among students of the 1914 class at the Connecticut Agricultural College. Because many genes contribute to height and tend to segregate independently of one another, the cumulative contribution of different combinations of alleles to height forms a *continuous* distribution of possible heights, in which the extremes are much rarer than the intermediate values. Variation can also arise due to environmental factors such as nutrition.

In pleiotropy, a single gene can affect more than one trait

Not only can more than one gene affect a single trait, but a single gene can affect more than one trait. Considering the complexity of biochemical pathways and the interdependent nature of organ systems in multicellular organisms, this should be no surprise.

An allele that has more than one effect on phenotype is said to be **pleiotropic.** The pioneering French geneticist Lucien Cuenot studied yellow fur in mice, a dominant trait, and found he was unable to obtain a pure-breeding yellow strain by crossing individual yellow mice with each other. Individuals homozygous for the yellow allele died, because the yellow allele was pleiotropic: One effect was yellow coat color, but another was a lethal developmental defect.

A pleiotropic allele may be dominant with respect to one phenotypic consequence (yellow fur) and recessive with respect to another (lethal developmental defect). Pleiotropic effects are difficult to predict, because a gene that affects one trait often performs other, unknown functions.

Pleiotropic effects are characteristic of many inherited disorders in humans, including cystic fibrosis and sickle cell anemia (discussed in chapter 13). In these disorders, multiple symptoms (phenotypes) can be traced back to a single gene defect. Cystic fibrosis patients exhibit clogged blood vessels, overly sticky mucus, salty sweat, liver and pancreas failure, and several other symptoms. It is often difficult to deduce the nature of the primary defect from the range of a gene's pleiotropic effects. As it turns out, all these symptoms of cystic fibrosis are pleiotropic effects of a single defect, a mutation in a gene that encodes a chloride ion transmembrane channel.

Genes may have more than two alleles

Mendel always looked at genes with two alternative alleles. Although any diploid individual can carry only two alleles for a

gene, there may be more than two alleles in a population. The example of ABO blood types in humans, described later on, involves an allelic series with three alleles.

If you think of a gene as a sequence of nucleotides in a DNA molecule, then the number of possible alleles is huge because even a single nucleotide change could produce a new allele. In reality, the number of alleles possible for any gene is constrained, but usually more than two alleles exist for any gene in an outbreeding population. The dominance relationships of these alleles cannot be predicted, but can be determined by observing the phenotypes for the various heterozygous combinations.

Dominance is not always complete

Mendel's idea of dominant and recessive traits can seem hard to explain in terms of modern biochemistry. For example, if a recessive trait is caused by the loss of function of an enzyme encoded by the recessive allele, then why should a heterozygote, with only half the activity of this enzyme, have the same appearance as a homozygous dominant individual?

The answer is that enzymes usually act in pathways and not alone. These pathways, as you have seen in earlier chapters, can be highly complex in terms of inputs and outputs, and they can sometimes tolerate large reductions in activity of single enzymes in the pathway without reductions in the level of the end-product. When this is the case, complete dominance will be observed; however, not all genes act in this way.

Incomplete dominance

In **incomplete dominance,** the heterozygote is intermediate in appearance between the two homozygotes. For example, in a cross between red- and white-flowering Japanese four o'clocks, described in figure 12.12, all the F_1 offspring have pink flowers—indicating that neither red nor white flower color was dominant. Looking only at the F_1, we might conclude that this is a case of blending inheritance. But when two of the F_1 pink flowers are crossed, they produce red-, pink-, and white-flowered plants in a 1:2:1 ratio. In this case the phenotypic ratio is the same as the genotypic ratio because all three genotypes can be distinguished.

Codominance

Most genes in a population possess several different alleles, and often no single allele is dominant; instead, each allele has its own effect, and the heterozygote shows some aspect of the phenotype of both homozygotes. The alleles are said to be **codominant.** Codominance can be distinguished from incomplete dominance by the appearance of the heterozygote. In incomplete dominance, the heterozygote is intermediate between the two homozygotes, whereas in codominance, some aspect of both alleles is seen in the heterozygote. One of the clearest human examples is found in the human blood groups.

The different phenotypes of human blood groups are based on the response of the immune system to proteins on the surface of red blood cells. In homozygotes a single type of protein is found on the surface of cells, and in heterozygotes, two kinds of protein are found, leading to codominance.

The human ABO blood group system

The gene that determines ABO blood types encodes an enzyme that adds sugar molecules to proteins on the surface of red blood cells. These sugars act as recognition markers for the immune system (see chapter 52). The gene that encodes the enzyme, designated *I*, has three common alleles: I^A, whose product adds galactosamine; I^B, whose product adds galactose; and *i*, which codes for a protein that does not add a sugar.

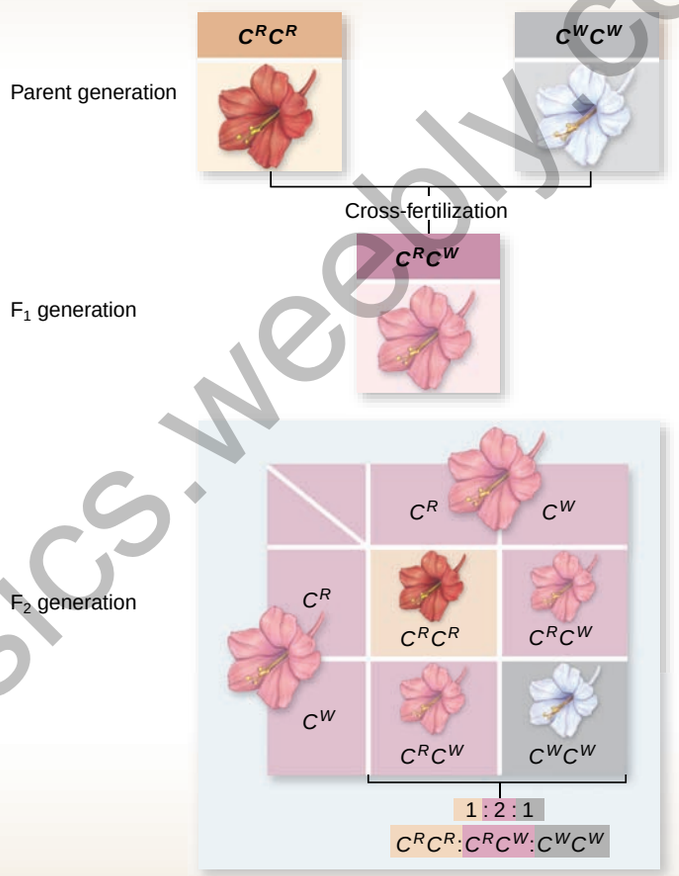
The three alleles of the *I* gene can be combined to produce six different genotypes. An individual heterozygous for the I^A and

SCIENTIFIC THINKING

Hypothesis: *The pink F_1 observed in a cross of red and white Japanese four o'clock flowers is due to failure of dominance and is not an example of blending inheritance.*

Prediction: *If pink F_1 are self-crossed, they will yield progeny the same as the Mendelian monohybrid genotypic ratio. This would be 1 red: 2 pink: 1 white.*

Test: *Perform the cross and count progeny.*



Result: *When this cross is performed, the expected outcome is observed.*

Conclusion: *Flower color in Japanese four o'clock plants exhibits incomplete dominance.*

Further Experiments: *How many offspring would you need to count to be confident in the observed ratio?*

Figure 12.12 Incomplete dominance. In a cross between a red-flowered (genotype C^RC^R) Japanese four o'clock and a white-flowered one (C^WC^W), neither allele is dominant. The heterozygous progeny have pink flowers and the genotype C^RC^W. If two of these heterozygotes are crossed, the phenotypes of their progeny occur in a ratio of 1:2:1 (red:pink:white).

I^B alleles produces both forms of the enzyme and exhibits both galactose and galactosamine on red blood cells. Because both alleles are expressed simultaneously in heterozygotes, the I^A and I^B alleles are codominant. Both I^A and I^B are dominant over the *i* allele, because both I^A and I^B alleles lead to sugar addition, whereas the *i* allele does not. The different combinations of the three alleles produce four different phenotypes (figure 12.13):

1. Type A individuals add only galactosamine. They are either I^AI^A homozygotes or I^Ai heterozygotes (two genotypes).
2. Type B individuals add only galactose. They are either I^BI^B homozygotes or I^Bi heterozygotes (two genotypes).
3. Type AB individuals add both sugars and are I^AI^B heterozygotes (one genotype).
4. Type O individuals add neither sugar and are *ii* homozygotes (one genotype).

These four different cell-surface phenotypes are called the **ABO blood groups.**

A person's immune system can distinguish among these four phenotypes. If a type A individual receives a transfusion of type B blood, the recipient's immune system recognizes the "foreign" antigen (galactose) and attacks the donated blood cells, causing them to clump, or agglutinate. The same thing would happen if the donated blood is type AB. However, if the donated blood is type O, no immune attack occurs, because there are no galactose antigens.

In general, any individual's immune system can tolerate a transfusion of type O blood, and so type O is termed the "universal donor." Because neither galactose nor galactosamine is foreign to type AB individuals (whose red blood cells have both sugars), those individuals may receive any type of blood, and type AB is termed the "universal recipient." Nevertheless, matching blood is preferable for any transfusion.

Phenotypes may be affected by the environment

Another assumption, implicit in Mendel's work, is that the environment does not affect the relationship between genotype and phenotype. For example, the soil in the abbey yard where Mendel performed his experiments was probably not uniform, and yet its possible effect on the expression of traits was ignored. But in reality, although the expression of genotype produces phenotype, the environment can affect this relationship.

Environmental effects are not limited to the external environment. For example, the alleles of some genes encode heat-sensitive products, that are affected by differences in internal body temperature. The *ch* allele in Himalayan rabbits and Siamese cats encodes a heat-sensitive version of the enzyme tyrosinase, which as you may recall is involved in albinism (figure 12.14). The Ch version of the enzyme is inactivated at temperatures above about 33°C. At the surface of the torso and head of these animals, the temperature is above 33°C and tyrosinase is inactive, producing a whitish coat. At the extremities, such as the tips of the ears and tail, the temperature is usually below 33°C and the enzyme is active, allowing production of melanin that turns the coat in these areas a dark color.

Inquiry question

Many studies of identical twins separated at birth have revealed phenotypic differences in their development (height, weight, etc.). If these are identical twins, can you propose an explanation for these differences?

In epistasis, interactions of genes alter genetic ratios

The last simplistic assumption in Mendel's model is that the products of genes do not interact. But the products of genes may not act independently of one another, and the interconnected behavior of gene products can change the ratio expected by independent assortment, even if the genes are on different chromosomes that do exhibit independent assortment.

Alleles	Blood Type	Sugars Exhibited	Donates and Receives
I^AI^A, I^Ai (I^A dominant to *i*)	A	Galactosamine	Receives A and O Donates to A and AB
I^BI^B, I^Bi (I^B dominant to *i*)	B	Galactose	Receives B and O Donates to B and AB
I^AI^B (codominant)	AB	Both galactose and galactosamine	Universal receiver Donates to AB
ii (*i* is recessive)	O	None	Receives O Universal donor

Figure 12.13 ABO blood groups illustrate both codominance and multiple alleles. There are three alleles of the *I* gene: I^A, I^B, and *i*. I^A and I^B are both dominant to *i* (see types A and B), but codominant to each other (see type AB). The genotypes that give rise to each blood type are shown with the associated phenotypes in terms of sugars added to surface proteins and the body's reaction after a blood transfusion.

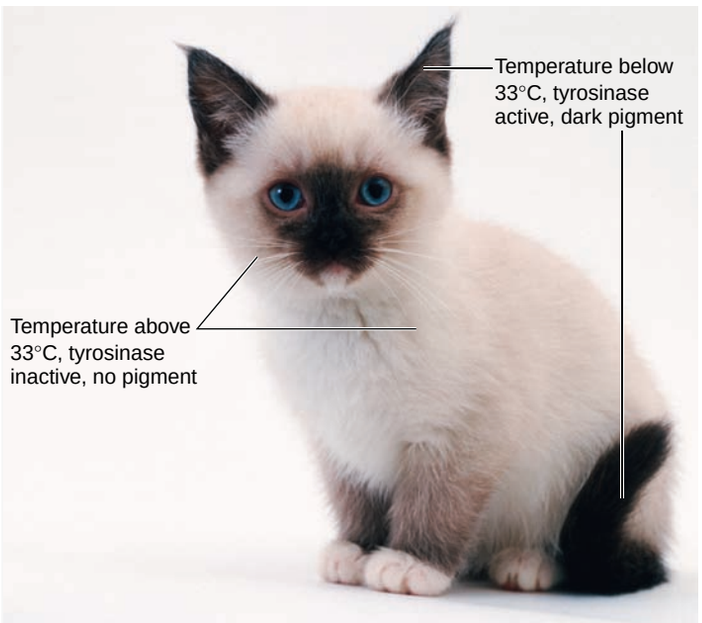


Figure 12.14 Siamese cat. The pattern of coat color is due to an allele that encodes a temperature-sensitive form of the enzyme tyrosinase.

Given the interconnected nature of metabolism, it should not come as a surprise that many gene products are not independent. Genes that act in the same metabolic pathway, for example, should show some form of dependence at the level of function. In such cases, the ratio Mendel would predict is not readily observed, but it is still there in an altered form.

In the tests of Mendel's ideas that followed the rediscovery of his work, scientists had trouble obtaining Mendel's simple ratios, particularly with dihybrid crosses. Sometimes, it was not possible to identify successfully each of the four phenotypic classes expected, because two or more of the classes looked alike.

An example of this comes from the analysis of particular varieties of corn, *Zea mays*. Some commercial varieties exhibit a purple pigment called anthocyanin in their seed coats, whereas others do not. In 1918, geneticist R. A. Emerson crossed two true-breeding corn varieties, each lacking anthocyanin pigment. Surprisingly, all of the F_1 plants produced purple seeds.

When two of these pigment-producing F_1 plants were crossed to produce an F_2 generation, 56% were pigment producers and 44% were not. This is clearly not what Mendel's ideas would lead us to expect. Emerson correctly deduced that two genes were involved in producing pigment, and that the second cross had thus been a dihybrid cross. According to Mendel's theory, gametes in a dihybrid cross could combine in 16 equally possible ways—so the puzzle was to figure out how these 16 combinations could occur in the two phenotypic groups of progeny. Emerson multiplied the fraction that were pigment producers (0.56) by 16 to obtain 9, and multiplied the fraction that lacked pigment (0.44) by 16 to obtain 7. Emerson therefore had a *modified ratio* of 9:7 instead of the usual 9:3:3:1 ratio (figure 12.15).

This modified ratio is easily rationalized by considering the function of the products encoded by these genes. When gene products act sequentially, as in a biochemical pathway, an allele expressed as a defective enzyme early in the pathway blocks the flow of material through the rest of the pathway. In this case, it is impossible to judge whether the later steps of the pathway are functioning properly. This type of gene interaction, in which one gene can interfere with the expression of another, is the basis of the phenomenon called **epistasis.**

The pigment anthocyanin is the product of a two-step biochemical pathway:

$$\text{starting molecule (colorless)} \xrightarrow{\text{enzyme 1}} \text{intermediate (colorless)} \xrightarrow{\text{enzyme 2}} \text{anthocyanin (purple)}$$

To produce pigment, a plant must possess at least one functional copy of each enzyme's gene. The dominant alleles encode functional enzymes, and the recessive alleles encode nonfunctional enzymes. Of the 16 genotypes predicted by random assortment, 9 contain at least one dominant allele of both genes; they therefore produce purple progeny. The remaining 7 genotypes lack dominant alleles at *either or both* loci (3 + 3 + 1 = 7) and so produce colorless progeny, giving the phenotypic ratio of 9:7 that Emerson observed (see figure 12.15).

You can see that although this ratio is not the expected dihybrid ratio, it is a modification of the expected ratio.

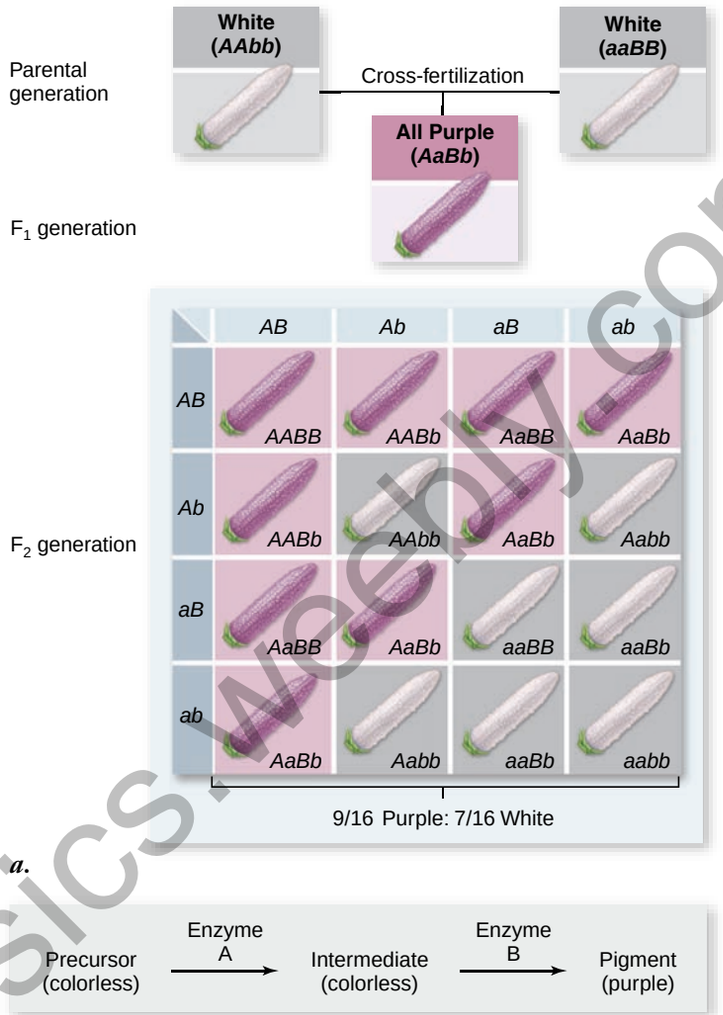


Figure 12.15 How epistasis affects grain color.
a. Crossing some white varieties of corn yields an all purple F_1. Self-crossing the F_1 yields 9 purple:7 white. This can be explained by the presence of two genes, each encoding an enzyme necessary for the production of purple pigment. Unless both enzymes are active (genotype is *A_B_*), no pigment is expressed. ***b.*** The biochemical pathway for pigment production with enzymes encoded by *A* and *B* genes.

Learning Outcomes Review 12.6

Mendel's model assumes that each trait is specified by one gene with only two alleles, no environmental effects alter a trait, and gene products act independently. All of these prove to be oversimplifications. Traits produced by the action of multiple genes (polygenic inheritance) have continuous variation. One gene can affect more than one trait (pleiotropy). Genes may have more than two alleles, and these may not show simple dominance. In incomplete dominance, the heterozygote is intermediate between the two homozygotes, and in codominance the heterozygote shows aspects of both homozygotes, both of which alter the monohybrid ratio. The action of genes is not always independent, which can result in modified dihybrid ratios.

- ***In the cross in figure 12.15, what proportion of F_2 will be white because they are homozygous recessive for one of the two genes?***

Chapter Review

12.1 The Mystery of Heredity

Early plant biologists produced hybrids and saw puzzling results.

Plant breeders noticed that some forms of a trait can disappear in one generation only to reappear later, that is, they segregate rather than blend.

Mendel used mathematics to analyze his crosses.

Mendel's experiments involved reciprocal crosses between true-breeding pea varieties followed by one or more generations of self-fertilization. His mathematical analysis of experimental results led to the present model of inheritance.

12.2 Monohybrid Crosses: The Principle of Segregation (see figure 12.5)

The F_1 generation exhibits only one of two traits, without blending.

Mendel called the trait visible in the F_1 the dominant trait; the other he termed recessive.

The F_2 generation exhibits both traits in a 3:1 ratio.

When F_1 plants are self-fertilized, the F_2 shows a consistent ratio of 3 dominant:1 recessive. We call this 3:1 ratio the Mendelian monohybrid ratio.

The 3:1 ratio is actually 1:2:1.

Mendel then examined the F_2 and found the recessive F_2 plants always bred true, but only one out of three dominant F_2 bred true. This means the 3:1 ratio is actually 1 true-breeding dominant:2 non-true-breeding dominant:1 recessive.

Mendel's Principle of Segregation explains monohybrid observations.

Traits are determined by discrete factors we now call genes. These exist in alternative forms we call alleles. Individuals carrying two identical alleles for a gene are said to be homozygous, and individuals carrying different alleles are said to be heterozygous. The genotype is the entire set of alleles of all genes possessed by an individual. The phenotype is the individual's appearance due to these alleles.

The Principle of Segregation states that during gamete formation, the two alleles of a gene separate (segregate). Parental alleles then randomly come together to form the diploid zygote. The physical basis of segregation is the separation of homologues during anaphase of meiosis I.

The Punnett square allows symbolic analysis.

Punnett squares are formed by placing the gametes from one parent along the top of the square with the gametes from the other parent along the side. Zygotes formed from gamete combinations form the blocks of the square (see figure 12.6).

Some human traits exhibit dominant/recessive inheritance.

Certain human traits have been found to have a Mendelian basis (see table 12.1). Inheritance patterns in human families can be analyzed and inferred using a pedigree diagram of earlier generations.

12.3 Dihybrid Crosses: The Principle of Independent Assortment (see figure 12.9)

Traits in a dihybrid cross behave independently.

If parents differing in two traits are crossed, the F_1 will be all dominant. Each F_1 parent can produce four different gametes that can be combined to produce 16 possible outcomes in the F_2. This yields a phenotypic ratio of 9:3:3:1 of the four possible phenotypes.

Mendel's Principle of Independent Assortment explains dihybrid results.

The Principle of Independent Assortment states that different traits segregate independently of one another. The physical basis of independent assortment is the independent behavior of different pairs of homologous chromosomes during meiosis I.

12.4 Probability: Predicting the Results of Crosses

Two probability rules help predict monohybrid cross results.

The rule of addition states that the probability of two independent events occurring is the sum of their individual probabilities. The rule of multiplication, or product rule, states that the probability of two independent events *both* occurring is the product of their individual probabilities.

Dihybrid cross probabilities are based on monohybrid cross probabilities.

A dihybrid cross is essentially two independent monohybrid crosses. The product rule applies and can be used to predict the cross's outcome.

12.5 The Testcross: Revealing Unknown Genotypes (see figure 12.10)

In a testcross, an unknown genotype is crossed with a homozygous recessive genotype. The F_1 offspring will all be the same if the unknown genetoype is homozygous dominant. The F_1 offspring will exhibit a 1:1 dominant:recessive ratio if the unknown genotype is heterozygous.

12.6 Extensions to Mendel

In polygenic inheritance, more than one gene can affect a single trait.

Many traits, such as human height, are due to multiple additive contributions by many genes, resulting in continuous variation.

In pleiotropy, a single gene can affect more than one trait.

A pleiotropic effect occurs when an allele affects more than one trait. These effects are difficult to predict.

Genes may have more than two alleles.

There may be more than two alleles of a gene in a population. Given the possible number of DNA sequences, this is not surprising.

Dominance is not always complete.

In incomplete dominance the heterozygote exhibits an intermediate phenotype; the monohybrid genotypic and phenotypic ratios are the same (see figure 12.12). Codominant alleles each contribute to the phenotype of a heterozygote.

Phenotypes may be affected by the environment.

Genotype determines phenotype, but the environment will have an effect on this relationship. Environment means both external and internal factors. For example, in Siamese cats, a temperature-sensitive enzyme produces more pigment in the colder peripheral areas of the body.

In epistasis, interactions of genes alter genetic ratios.

Genes encoding enzymes that act in a single biochemical pathway are not independent. In corn, anthocyanin pigment production requires the action of two enzymes. Doubly heterozygous individuals for these enzymes yield a 9:7 ratio when self-crossed (see figure 12.15).

Review Questions

UNDERSTAND

1. What property distinguished Mendel's investigation from previous studies?
   a. Mendel used true-breeding pea plants.
   b. Mendel quantified his results.
   c. Mendel examined many different traits.
   d. Mendel examined the segregation of traits.
2. The F_1 generation of the monohybrid cross purple (*PP*) × white (*pp*) flower pea plants should
   a. all have white flowers.
   b. all have a light purple or blended appearance.
   c. all have purple flowers.
   d. have (¾) purple flowers, and ¼ white flowers.
3. The F_1 plants from the previous question are allowed to self-fertilize. The phenotypic ratio for the F_2 should be
   a. all purple.
   b. 1 purple:1 white.
   c. 3 purple:1 white.
   d. 3 white:1 purple.
4. Which of the following is *not* a part of Mendel's five-element model?
   a. Traits have alternative forms (what we now call alleles).
   b. Parents transmit discrete traits to their offspring.
   c. If an allele is present it will be expressed.
   d. Traits do not blend.
5. An organism's _____ is/are determined by its _____.
   a. genotype; phenotype
   b. phenotype; genotype
   c. alleles; phenotype
   d. genes; alleles
6. Phenotypes like height in humans, which show a continuous distribution, are usually the result of
   a. an alteration of dominance for multiple alleles of a single gene.
   b. the presence of multiple alleles for a single gene.
   c. the action of one gene on multiple phenotypes.
   d. the action of multiple genes on a single phenotype.

APPLY

1. A dihybrid cross between a plant with long smooth leaves and a plant with short hairy leaves produces a long smooth F_1. If this F_1 is allowed to self-cross to produce an F_2, what would you predict for the ratio of F_2 phenotypes?
   a. 9 long smooth:3 long hairy:3 short hairy:1 short smooth
   b. 9 long smooth:3 long hairy:3 short smooth:1 short hairy
   c. 9 short hairy:3 long hairy:3 short smooth:1 long smooth
   d. 1 long smooth:1 long hairy:1 short smooth:1 short hairy
2. Consider a long smooth F_2 plant from the previous question. This plant's genotype
   a. must be homozygous for both long alleles and hairy alleles.
   b. must be heterozygous at both the leaf length gene, and the leaf hair gene.
   c. can only be inferred by another cross.
   d. cannot be determined by any means.
3. What is the probability of obtaining an individual with the genotype *bb* from a cross between two individuals with the genotype *Bb*?
   a. ½
   b. ¼
   c. ⅛
   d. 0
4. What is the probability of obtaining an individual with the genotype *CC* from a cross between two individuals with the genotypes *CC* and *Cc*?
   a. ½
   b. ¼
   c. ⅛
   d. $\frac{1}{16}$
5. You discover a new variety of plant with color varieties of purple and white. When you intercross these, the F_1 is a lighter purple. You consider that this may be an example of blending and self-cross the F_1. If Mendel is correct, what would you predict for the F_2?
   a. 1 purple:2 white:1 light purple
   b. 1 white:2 purple:1 light purple
   c. 1 purple:2 light purple:1 white
   d. 1 light purple:2 purple:1 white
6. Mendel's model assumes that each trait is determined by a single factor with alternate forms. We now know that this is too simplistic and that
   a. a single gene may affect more than one trait.
   b. a single trait may be affected by more than one gene.
   c. a single gene always affects only one trait, but traits may be affected by more than one gene.
   d. a single gene can affect more than one trait, and traits may be affected by more than one gene.

SYNTHESIZE

1. Create a Punnett square for the following crosses and use this to predict phenotypic ratio for dominant and recessive traits. Dominant alleles are indicated by uppercase letters and recessive are indicated by lowercase letters. For parts b and c, predict ratios using probability and the product rule.
   a. A monohybrid cross between individuals with the genotype *Aa* and *Aa*
   b. A dihybrid cross between two individuals with the genotype *AaBb*
   c. A dihybrid cross between individuals with the genotype *AaBb* and *aabb*
2. Explain how the events of meiosis can explain both segregation and independent assortment.
3. In mice, there is a yellow strain that when crossed yields 2 yellow:1 black. How could you explain this observation? How could you test this with crosses?
4. In mammals, a variety of genes affect coat color. One of these is a gene with mutant alleles that results in the complete loss of pigment, or albinism. Another controls the type of dark pigment with alleles that lead to black or brown colors. The albinistic trait is recessive, and black is dominant to brown. Two black mice are crossed and yield 9 black:4 albino:3 brown. How would you explain these results?

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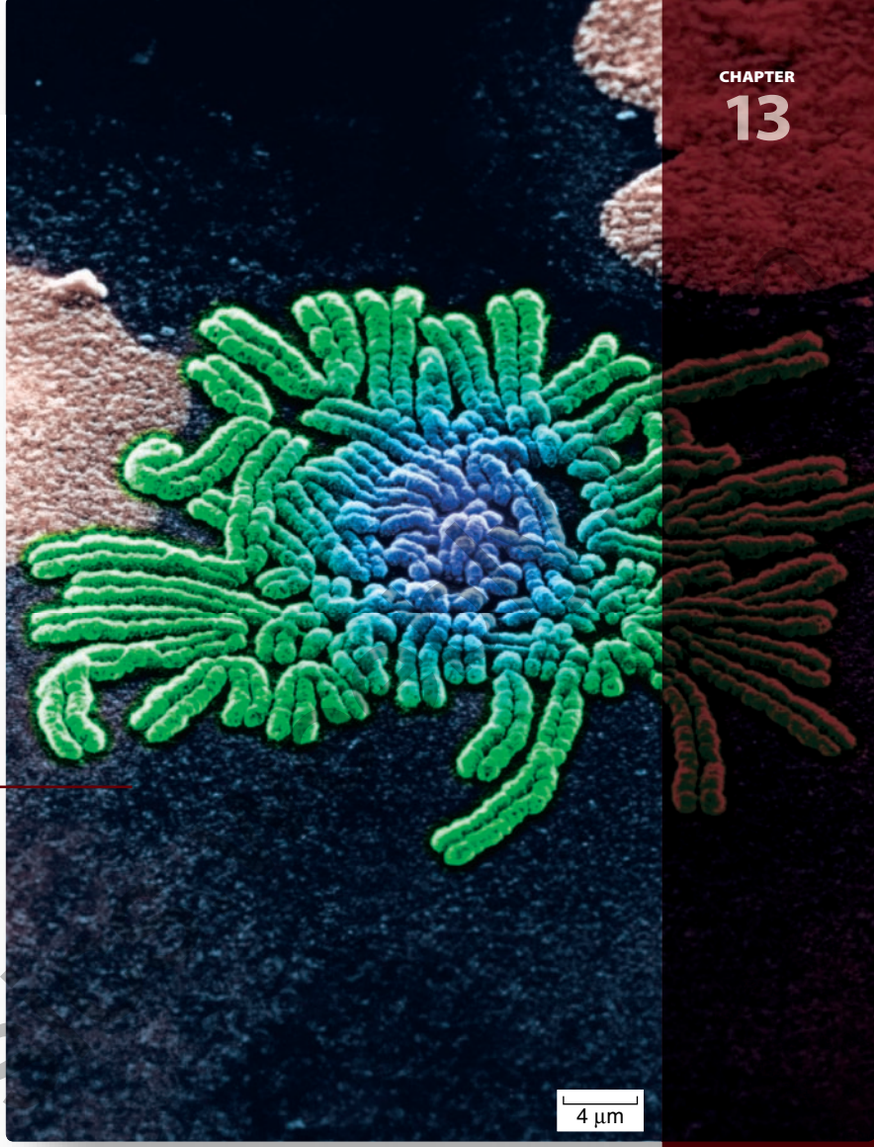
Chapter 13

Chromosomes, Mapping, and the Meiosis–Inheritance Connection



Chapter Outline

Introduction

Mendel's experiments opened the door to understanding inheritance, but many questions remained. In the early part of the 20th century, we did not know the nature of the factors whose behavior Mendel had described. The next step, which involved many researchers in the early part of the century, was uniting information about the behavior of chromosomes, seen in the picture, and the inheritance of traits. The basis for Mendel's principles of segregation and independent assortment lie in events that occur during meiosis.

The behavior of chromosomes during meiosis not only explains Mendel's principles, but leads to new and different approaches to the study of heredity. The ability to construct genetic maps is one of the most powerful tools of classical genetic analysis. The tools of genetic mapping developed in flies and other organisms in combination with information from the Human Genome Project now allow us to determine the location of genes and isolate those that are involved in genetic diseases.

13.1 Sex Linkage and the Chromosomal Theory of Inheritance

Learning Outcomes

1. *Describe sex-linked inheritance in fruit flies.*
2. *Explain the evidence for genes being on chromosomes.*

A central role for chromosomes in heredity was first suggested in 1900 by the German geneticist Carl Correns, in one of the papers announcing the rediscovery of Mendel's work. Soon after, observations that similar chromosomes paired with one another during meiosis led directly to the **chromosomal theory of inheritance,** first formulated by the American Walter Sutton in 1902.

Morgan correlated the inheritance of a trait with sex chromosomes

In 1910, Thomas Hunt Morgan, studying the fruit fly *Drosophila melanogaster*, discovered a mutant male fly with white eyes instead of red (figure 13.1).

Morgan immediately set out to determine whether this new trait would be inherited in a Mendelian fashion. He first crossed the mutant male to a normal red-eyed female to see whether the red-eyed or white-eyed trait was dominant. All of the F_1 progeny had red eyes, so Morgan concluded that red eye color was dominant over white.

The F_1 cross

Following the experimental procedure that Mendel had established long ago, Morgan then crossed the red-eyed flies from the F_1 generation with each other. Of the 4252 F_2 progeny Morgan examined, 782 (18%) had white eyes. Although the ratio of red eyes to white eyes in the F_2 progeny was greater than 3:1, the results of the cross nevertheless provided clear evidence that eye color segregates. However, something about the outcome was strange and totally unpredicted by Mendel's theory—*all of the white-eyed F_2 flies were males!* (Figure 13.2)

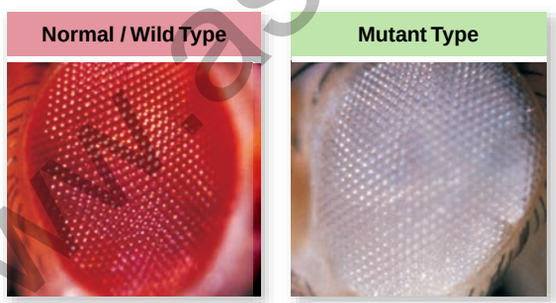


Figure 13.1 Red-eyed (wild type) and white-eyed (mutant) *Drosophila*. Mutations are heritable alterations in genetic material. By studying the inheritance pattern of white and red alleles (located on the X chromosome), Morgan first demonstrated that genes are on chromosomes.

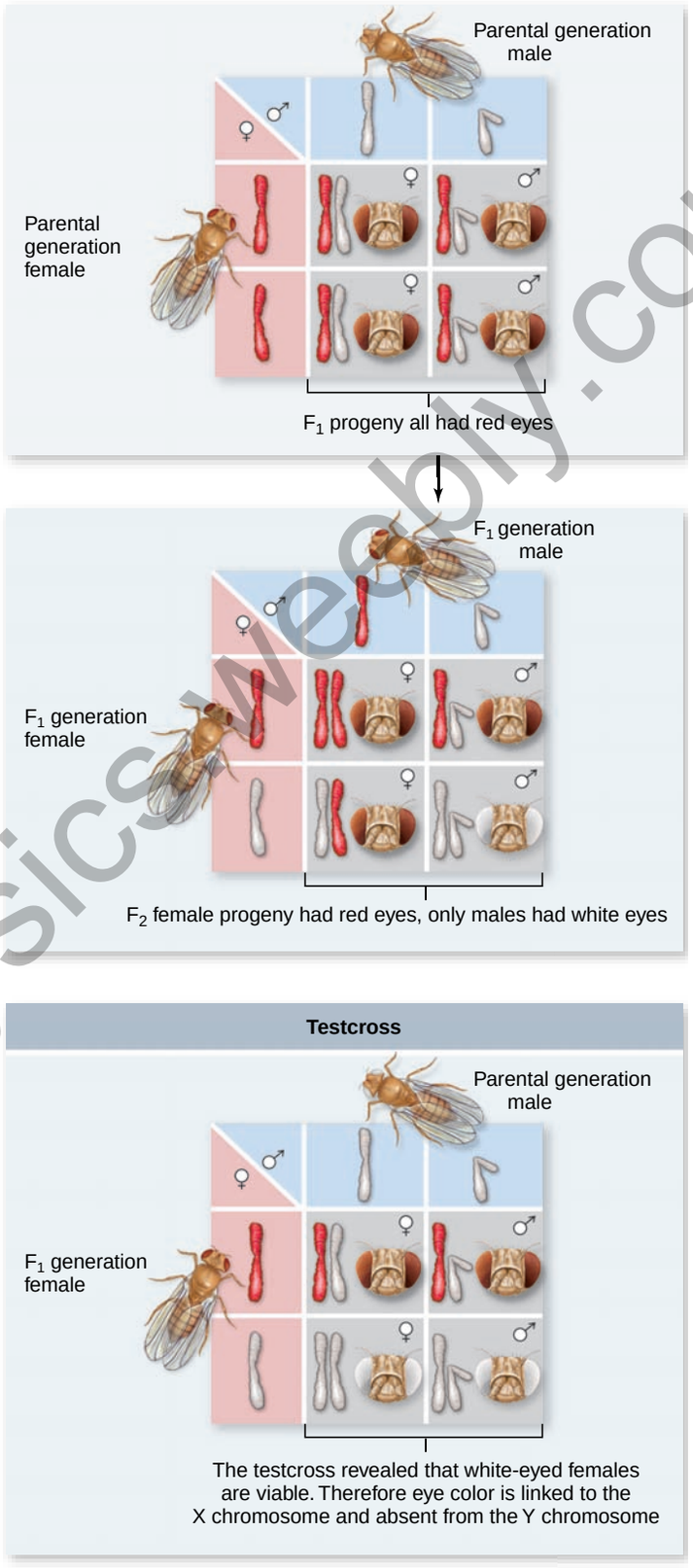


Figure 13.2 The chromosomal basis of sex linkage. White-eyed male flies are crossed to red-eyed females. The F_1 flies all have red eyes, as expected for a recessive white-eye allele. In the F_2, all of the white-eyed flies are male because the Y chromosome lacks the white-eye (*white*) gene. Inheritance of the sex chromosomes correlates with eye color, showing the *white* gene is on the X chromosome.

The testcross

Morgan sought an explanation for this result. One possibility was simply that white-eyed female flies don't exist; such individuals might not be viable for some unknown reason. To test this idea, Morgan testcrossed the female F_1 progeny with the original white-eyed male. He obtained white-eyed and red-eyed flies of both sexes in a 1:1:1:1 ratio, just as Mendel's theory had predicted (figure 13.2). Therefore, white-eyed female flies are viable. Given that white-eyed females can exist, Morgan turned to the nature of the chromosomes in males and females for an explanation.

The gene for eye color lies on the X chromosome

In *Drosophila*, the sex of an individual is determined by the number of copies it has of a particular chromosome, the **X chromosome.** Observations of *Drosophila* chromosomes revealed that female flies have two X chromosomes, but male flies have only one. In males, the single X chromosome pairs in meiosis with a dissimilar partner called the **Y chromosome.** These two chromosomes are termed **sex chromosomes** because of their association with sex.

During meiosis, a female produces only X-bearing gametes, but a male produces both X-bearing and Y-bearing gametes. When fertilization involves an X sperm, the result is an XX zygote, which develops into a female; when fertilization involves a Y sperm, the result is an XY zygote, which develops into a male.

The solution to Morgan's puzzle is that the gene causing the white-eye trait in *Drosophila* resides only on the X chromosome—it is absent from the Y chromosome. (We now know that the Y chromosome in flies carries almost no functional genes.) A trait determined by a gene on the X chromosome is said to be **sex-linked,** or X-linked, because it is associated with the sex of the individual. Knowing the white-eye trait is recessive to the red-eye trait, we can now see that Morgan's result was a natural consequence of the Mendelian segregation of chromosomes (see figure 13.2).

Morgan's experiment was one of the most important in the history of genetics because it presented the first clear evidence that the genes determining Mendelian traits do indeed reside on the chromosomes, as Sutton had proposed. Mendelian traits segregate in genetic crosses because homologues separate during gamete formation.

Learning Outcomes Review 13.1

Morgan showed that the trait for white eyes in *Drosophila* segregated with the sex of offspring. X and Y chromosomes also segregate with sex, this correlates the behavior of a trait with the behavior of chromosomes. This finding supported the chromosomal theory of inheritance, which states that traits are carried on chromosomes.

- ***What are the expectations for a cross of white-eyed females to red-eyed males?***

13.2 Sex Chromosomes and Sex Determination

Learning Outcomes

1. ***Describe the relationship between sex chromosomes and sex determination.***
2. ***Explain dosage compensation in mammals and its genetic consequences.***

The structure and number of sex chromosomes vary in different species (table 13.1). In the fruit fly, *Drosophila*, females are XX and males XY, which is also the case for humans and other mammals. However, in birds, the male has two Z chromosomes, and the female has a Z and a W chromosome. Some insects, such as grasshoppers, have no Y chromosome—females are XX and males are characterized as XO (O indicates the absence of a chromosome).

In humans, the Y chromosome generally determines maleness

In chapter 10, you learned that humans have 46 chromosomes (23 pairs). Twenty-two of these pairs are perfectly matched in both males and females and are called **autosomes.** The remaining pair are the sex chromosomes: XX in females, and XY in males.

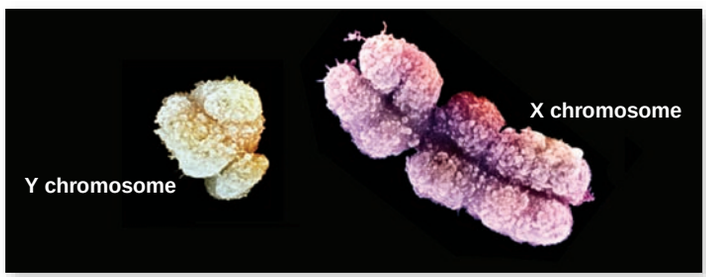


TABLE 13.1 Sex Determination in Some Organisms

	Female	Male
Humans, *Drosophila*	XX	XY
Birds	ZW	ZZ
Grasshoppers	XX	XO
Honeybees	Diploid	Haploid

The Y chromosome in males is highly condensed. Because few genes on the Y chromosome are expressed, recessive alleles on a male's single X chromosome have no *active* counterpart on the Y chromosome.

The "default" setting in human embryonic development is for production of a female. Some of the active genes on the Y chromosome, notably the *SRY* gene, are responsible for the masculinization of genitalia and secondary sex organs, producing features associated with "maleness" in humans. Consequently, any individual with *at least one Y chromosome* is normally a male.

The exceptions to this rule actually provide support for this mechanism of sex determination. For example, movement of part of the Y chromosome to the X chromosome can cause otherwise XX individuals to develop as male. There is also a genetic disorder that causes a failure to respond to the androgen hormones (androgen insensitivity syndrome) that causes XY individuals to develop as female. Lastly, mutations in *SRY* itself can cause XY individuals to develop as females.

This form of sex determination seen in humans is shared among mammals, but is not universal in vertebrates. Among fishes and some species of reptiles, environmental factors can cause changes in the expression of this sex-determining gene, and thus in the sex of the adult individual.

Some human genetic disorders display sex linkage

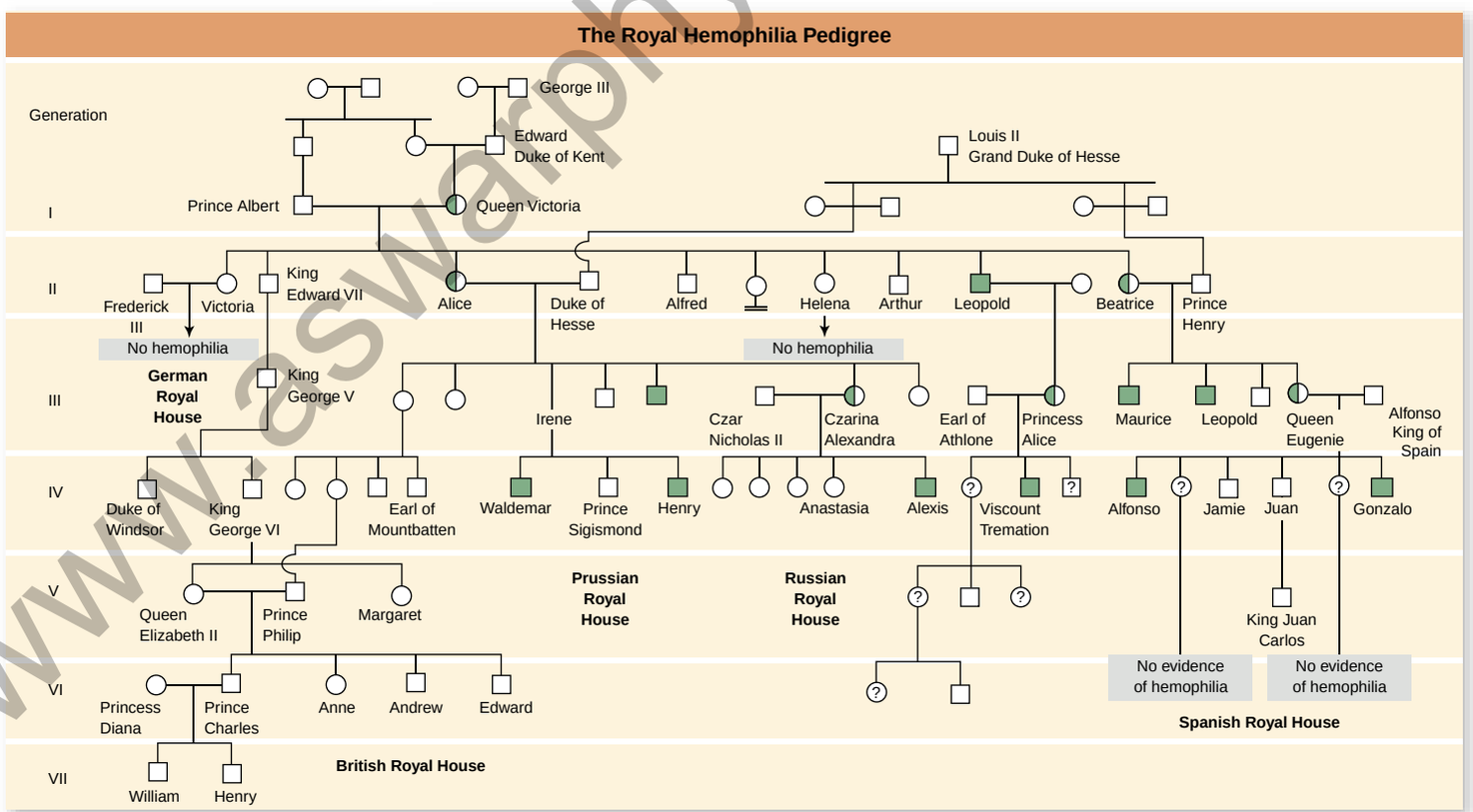
From ancient times, people have noted conditions that seem to affect males to a greater degree than females. Red-green color blindness is one well-known condition that is more common in males because the gene affected is carried on the X chromosome.

Another example is hemophilia, a disease that affects a single protein in a cascade of proteins involved in the formation of blood clots. Thus, in an untreated hemophiliac, even minor cuts will not stop bleeding. This form of hemophilia is caused by an X-linked recessive allele; women who are heterozygous for the allele are asymptomatic carriers, and men who receive an X chromosome with the recessive allele exhibit the disease.

The allele for hemophilia was introduced into a number of different European royal families by Queen Victoria of England. Because these families kept careful genealogical records, we have an extensive pedigree for this condition. In the five generations after Victoria, ten of her male descendants have had hemophilia as shown in the pedigree in figure 13.3.

The Russian house of Romanov inherited this condition through Alexandra Feodorovna, a granddaughter of Queen

Figure 13.3 The royal hemophilia pedigree. Queen Victoria, shown at the bottom center of the photo, was a carrier for hemophilia. Two of Victoria's four daughters, Alice and Beatrice, inherited the hemophilia allele from Victoria. Two of Alice's daughters are standing behind Victoria (wearing feathered boas): Princess Irene of Prussia *(right)* and Alexandra *(left)*, who would soon become czarina of Russia. Both Irene and Alexandra were also carriers of hemophilia. From the pedigree, it is clear that Alice introduced hemophilia into the Russian and Prussian royal houses, and Victoria's daughter Beatrice introduced it into the Spanish royal house. Victoria's son Leopold, himself a victim, also transmitted the disorder in a third line of descent. Half-shaded symbols represent carriers with one normal allele and one defective allele; fully shaded symbols represent affected individuals.



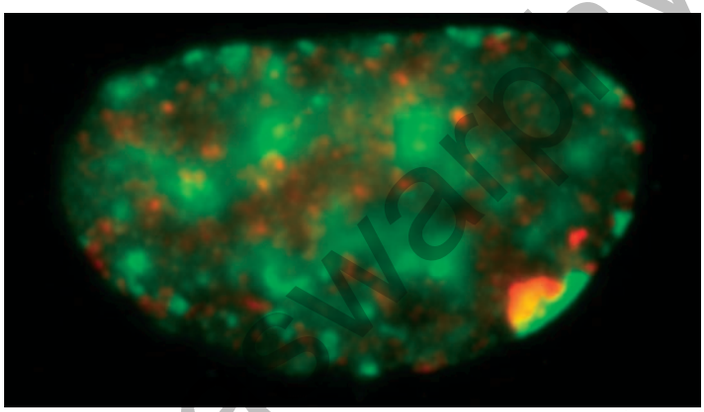
Victoria. She married Czar Nicholas II, and their only son, Alexis, was afflicted with the disease. The entire family was executed during the Russian revolution. (Recently, a woman who had long claimed to be Anastasia, a surviving daughter, was shown not to be a Romanov using modern genetic techniques to test her remains.)

Ironically, this condition has not affected the current British royal family, because Victoria's son Edward, who became King Edward VII, did not receive the hemophilia allele. All of the subsequent rulers of England are his descendants.

Dosage compensation prevents doubling of sex-linked gene products

Although males have only one copy of the X chromosome and females have two, female cells do not produce twice as much of the proteins encoded by genes on the X chromosome. Instead, one of the X chromosomes in females is inactivated early in embryonic development, shortly after the embryo's sex is determined. This inactivation is an example of **dosage compensation,** which ensures an equal level of expression from the sex chromosomes despite a differing number of sex chromosomes in males and females. (In *Drosophila*, by contrast, dosage compensation is achieved by increasing the level of expression on the male X chromosome.)

Which X chromosome is inactivated in females varies randomly from cell to cell. If a woman is heterozygous for a sex-linked trait, some of her cells will express one allele and some the other. The inactivated X chromosome is highly condensed, making it visible as an intensely staining **Barr body,** seen below, attached to the nuclear membrane.



X chromosome inactivation can lead to genetic mosaics

X chromosome inactivation to produce dosage compensation is not unique to humans but is true of all mammals. Females that are heterozygous for X chromosome alleles are **genetic mosaics:** Their individual cells may express different alleles, depending on which chromosome is inactivated.

One example is the calico cat, a female that has a patchy distribution of dark fur, orange fur, and white fur (figure 13.4). The dark fur and orange fur are due to heterozygosity for a gene on the X chromosome that determines pigment color. One allele results in dark fur, and another allele results in orange fur. Which of these colors is observed in any particular patch is due to inactivation of one X chromosome: If the chromosome containing the orange allele is inactivated, then the fur will be dark, and vice versa.

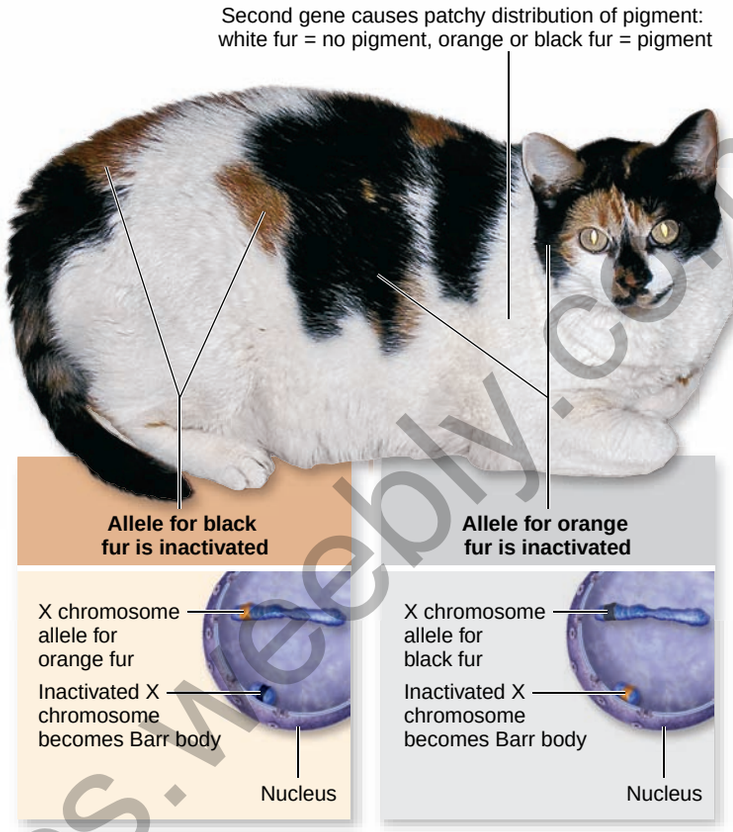


Figure 13.4 A calico cat. The cat is heterozygous for alleles of a coat color gene that produce either black fur or orange fur. This gene is on the X chromosome, so the different-colored fur is due to inactivation of one X chromosome. The patchy distribution and white color is due to a second gene that is epistatic to the coat color gene and thus masks its effects.

The patchy distribution of color, and the presence of white fur, is due to a second gene that is epistatic to the fur color gene (see chapter 12). That is, the presence of this second gene produces a patchy distribution of pigment, with some areas totally lacking pigment. In the areas that lack pigment, the effect of either fur color allele is masked. Thus, in this one animal we can see an excellent example of both epistasis and X inactivation.

Learning Outcomes Review 13.2

Sex determination begins with the presence or absence of certain chromosomes termed the sex chromosomes. Additional factors may influence sex determination in different species. In humans, males are XY, and therefore they exhibit recessive traits for alleles on the X chromosome. In mammalian females, one X chromosome in each cell becomes inactivated to balance the levels of gene expression. This random inactivation can lead to genetic mosaics.

- ***Would you expect an XXX individual to be viable? If so, would that individual be male or female?***

13.3 Exceptions to the Chromosomal Theory of Inheritance

Learning Outcomes

1. *Explain why the presence of DNA in organelles leads to non-Mendelian inheritance.*
2. *Describe the inheritance pattern of organelle DNA.*

Although the chromosomal theory explains most inheritance, there are exceptions. Primarily, these are due to the presence of DNA in organelle genomes, specifically in mitochondria and chloroplasts. Non-Mendelian inheritance via organelles was studied in depth by Ruth Sager, who in the face of universal skepticism constructed the first map of chloroplast genes in *Chlamydomonas*, a unicellular green alga, in the 1960s and 1970s.

Mitochondria and chloroplasts are not partitioned with the nuclear genome by the process of meiosis. Thus any trait that is due to the action of genes in these organelles will not show Mendelian inheritance.

Mitochondrial genes are inherited from the female parent

Organelles are usually inherited from only one parent, generally the mother. When a zygote is formed, it receives an equal contribution of the nuclear genome from each parent, but it gets all of its mitochondria from the egg cell, which contains a great deal more cytoplasm (and thus organelles). As the zygote divides, these original mitochondria divide as well and are partitioned randomly.

As a result, the mitochondria in every cell of an adult organism can be traced back to the original maternal mitochondria present in the egg. This mode of uniparental (one-parent) inheritance from the mother is called **maternal inheritance.**

In humans, the disease Leber's hereditary optic neuropathy (LHON) shows maternal inheritance. The genetic basis of this disease is a mutant allele for a subunit of NADH dehydrogenase. The mutant allele reduces the efficiency of electron flow in the electron transport chain in mitochondria (see chapter 7), in turn reducing overall ATP production. Some nerve cells in the optic system are particularly sensitive to reduction in ATP production, resulting in neural degeneration.

A mother with this disease will pass it on to all of her progeny, whereas a father with the disease will not pass it on to any of his progeny. Note that this condition differs from sex-linked inheritance because males and females are equally affected.

Chloroplast genes may also be passed on uniparentally

The inheritance pattern of chloroplasts is also usually maternal, although both paternal and biparental inheritance of chloroplasts may be observed in some species. Carl Correns first hypothesized in 1909 that chloroplasts were responsible for inheritance of variegation (mixed green and white leaves) in the plant commonly known as the four o'clock *(Mirabilis jalapa)*. The offspring exhibited the phenotype of the female parent, regardless of the male's phenotype.

In Sager's work on *Chlamydomonas*, resistance to the antibiotic streptomycin was shown to be transmitted via the chloroplast DNA from only the mt^+ mating type. The mt^- mating type does not contribute chloroplast DNA to the zygote formed by fusion of mt^+ and mt^- gametes.

Learning Outcomes Review 13.3

The genomes of mitochondria and chloroplasts divide independently of the nucleus. These organelles are carried in the cytoplasm of the egg cell, so any traits determined by these genomes are maternally inherited and thus do not follow Mendelian rules. In some species, however, chloroplasts may be passed on paternally or biparentally.

- ***How can you explain the lack of mt^- chloroplast DNA in* Chlamydomonas *zygotes from mt^- by mt^+ crosses?***

13.4 Genetic Mapping

Learning Outcomes

1. *Recognize that genes on the same chromosome may not assort independently.*
2. *Explain how recombination frequency is related to genetic distance.*
3. *Review how data from testcrosses is used to construct genetic maps.*

We have seen that Mendelian traits are determined by genes located on chromosomes and that the independent assortment of Mendelian traits reflects the independent assortment of chromosomes in meiosis. This is fine as far as it goes, but it is still incomplete. Of Mendel's seven traits in figure 12.4, six are on different chromosomes and two are on the same chromosome, yet all show independent assortment with one another. The two on the same chromosome should not behave the same as those that are on different chromosomes. In fact, organisms generally have many more genes that assort independently than the number of chromosomes. This means that independent assortment cannot be due only to the random alignment of chromosomes during meiosis.

Inquiry question

Mendel did not examine plant height and pod shape in his dihybrid crosses. The genes for these traits are very close together on the same chromosome. How would this have changed Mendel's results?

The solution to this problem is found in an observation that was introduced in chapter 11: the crossing over of homologues during meiosis. In prophase I of meiosis, homologues appear to physically exchange material by crossing over (figure 13.5). In chapter 11, you saw how this was part of the mechanism that allows homologues, and not sister chromatids, to disjoin at anaphase I.

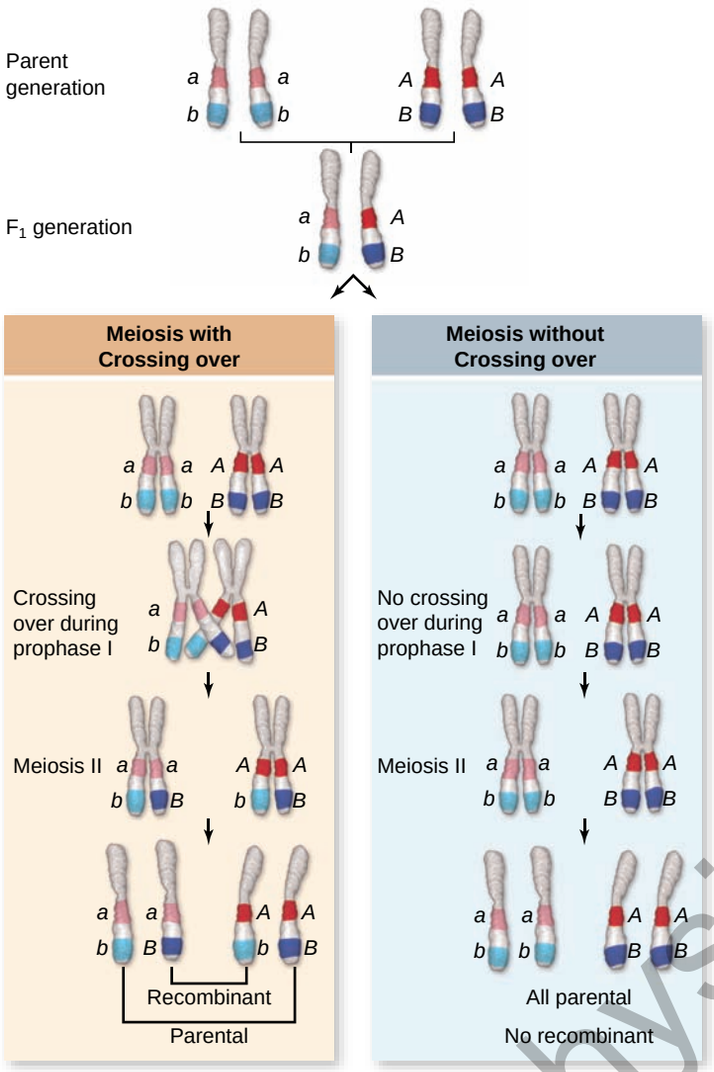


Figure 13.5 Crossing over exchanges alleles on homologues. When a crossover occurs between two loci, it leads to the production of recombinant chromosomes. If no crossover occurs, then the chromosomes will carry the parental combination of alleles.

Genetic recombination exchanges alleles on homologues

Consider a dihybrid cross performed using the Mendelian framework. Two true-breeding parents that each differ with respect to two traits are crossed, producing doubly heterozygous F_1 progeny. If the genes for the two traits are on a single chromosome, then during meiosis we would expect alleles for both loci to segregate together and produce only gametes that resemble the two parental types. But if a crossover occurs between the two loci, then each homologue would carry one allele from each parent and produce gametes that combine these parental traits (see figure 13.5). We call gametes with this new combination of alleles *recombinant* gametes as they are formed by recombining the parental alleles.

The first investigator to provide evidence for this was Morgan, who studied three genes on the X chromosome of *Drosophila*. He found an excess of parental types, which he explained as due to the genes all being on the X chromosome and therefore coinherited (inherited together). He went further, suggesting that the recombinant genotypes were due to crossing over between homologues during meiosis.

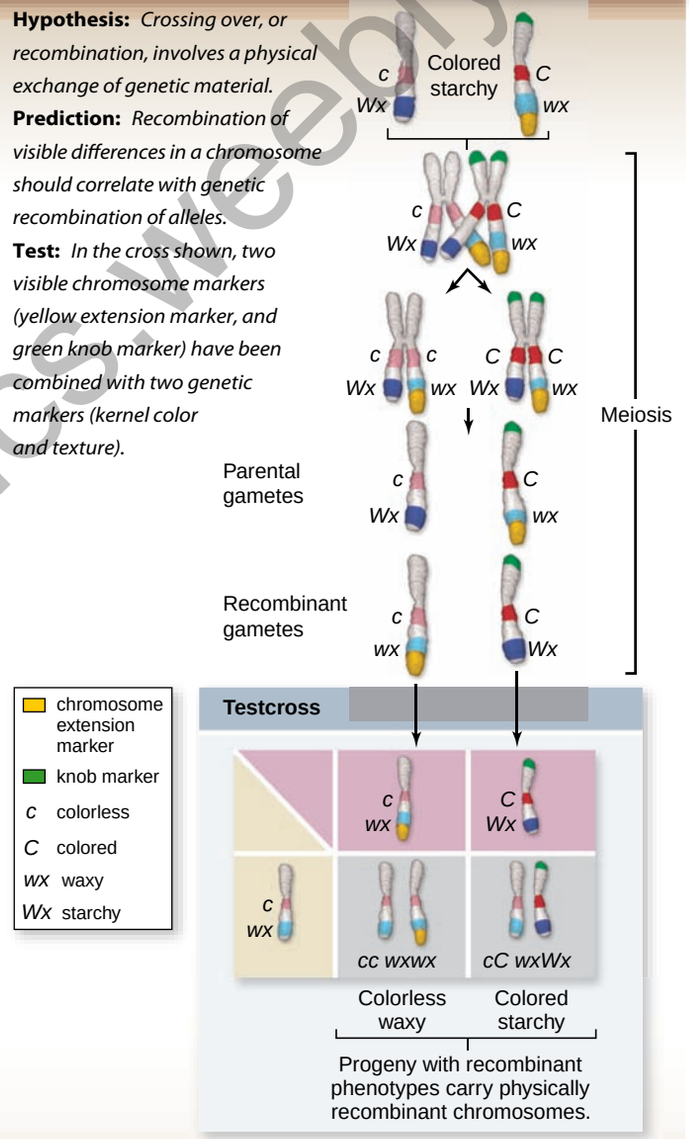
Experiments performed independently by Barbara McClintock and Harriet Creighton in maize and by Curt Stern in *Drosophila* provided evidence for this physical exchange of genetic material. The experiment done by Creighton and McClintock is detailed in figure 13.6. In this experiment, they used a chromosome with two alterations visible under a microscope: a knob on one end of the chromosome and an extension of the other end making it longer. In addition to these visible markers, this chromosome also carried a gene that determines kernel color (colored or colorless) and a gene that determines kernel texture (waxy or starchy).

SCIENTIFIC THINKING

Hypothesis: *Crossing over, or recombination, involves a physical exchange of genetic material.*

Prediction: *Recombination of visible differences in a chromosome should correlate with genetic recombination of alleles.*

Test: *In the cross shown, two visible chromosome markers (yellow extension marker, and green knob marker) have been combined with two genetic markers (kernel color and texture).*



Result: *Genetically recombinant progeny also have physically recombinant chromosomes.*

Conclusion: *A physical exchange of genetic material accompanied genetic recombination.*

Further Experiments: *This experiment was performed using maize. What other genetic model system would you use to test this?*

Figure 13.6 The Creighton and McClintock experiment.

The long chromosome, which also had the knob, carried the dominant colored allele for kernel color *(C)* and the recessive waxy allele for kernel texture *(wx)*. Heterozygotes were constructed with this chromosome paired with a visibly normal chromosome carrying the recessive colorless allele for kernel color *(c)* and the dominant starchy allele for kernel texture *(Wx)* (see figure 13.6). These plants appeared colored and starchy because they were heterozygous for both loci, and they were also heterozygous for the two visibly distinct chromosomes.

These plants, heterozygous for both chromosomal and genetic markers, were test crossed to colorless waxy plants with normal appearing chromosomes. The progeny were analyzed for both physical recombination (using a microscope to observe chromosome appearance) and genetic recombination (by examining the phenotype of progeny). The results were striking: All of the progeny that were genetically recombinant (appear colored starchy or colorless waxy) also now had only one of the chromosomal markers. That is, genetic recombination was accompanied by physical exchange of chromosomal material.

Recombination is the basis for genetic maps

The ability to map the location of genes on chromosomes using data from genetic crosses is one of the most powerful tools of genetics. The insight that allowed this technique, like many great insights, is so simple as to seem obvious in retrospect.

Morgan had already suggested that the frequency with which a particular group of recombinant progeny appeared was a reflection of the relative location of genes on the chromosome. An undergraduate in Morgan's laboratory, Alfred Sturtevant put this observation on a quantitative basis. Sturtevant reasoned that the frequency of recombination observed in crosses could be used as a measure of genetic distance. That is, as physical distance on a chromosome increases, so does the probability of recombination (crossover) occurring between the gene loci. Using this logic, the frequency of recombinant gametes produced is a measure of their distance apart on a chromosome.

Linkage data

To be able to measure recombination frequency easily, investigators used a testcross instead of intercrossing the F_1 progeny to produce an F_2 generation. In a testcross, as described earlier, the phenotypes of the progeny reflect the gametes produced by the doubly heterozygous F_1 individual. In the case of recombination, progeny that appear parental have not undergone crossover, and progeny that appear recombinant have experienced a crossover between the two loci in question (see figure 13.5).

When genes are close together, the number of recombinant progeny is much lower than the number of parental progeny, and the genes are defined on this basis as being **linked.** The number of recombinant progeny divided by total progeny gives a value defined as the **recombination frequency.** This value is converted to a percentage, and each 1% of recombination is termed a **map unit.** This unit has been named the centimorgan (cM) for T. H. Morgan, although it is also called simply a map unit (m.u.) as well.

Constructing maps

Constructing genetic maps then becomes a simple process of performing testcrosses with doubly heterozygous individuals and counting progeny to determine percent recombination. This is best shown with an example using a two-point cross.

Drosophila homozygous for two mutations, vestigial wings *(vg)* and black body *(b)*, are crossed to flies homozygous for the wild type, or normal alleles, of these genes *(vg^+ b^+)*. The doubly

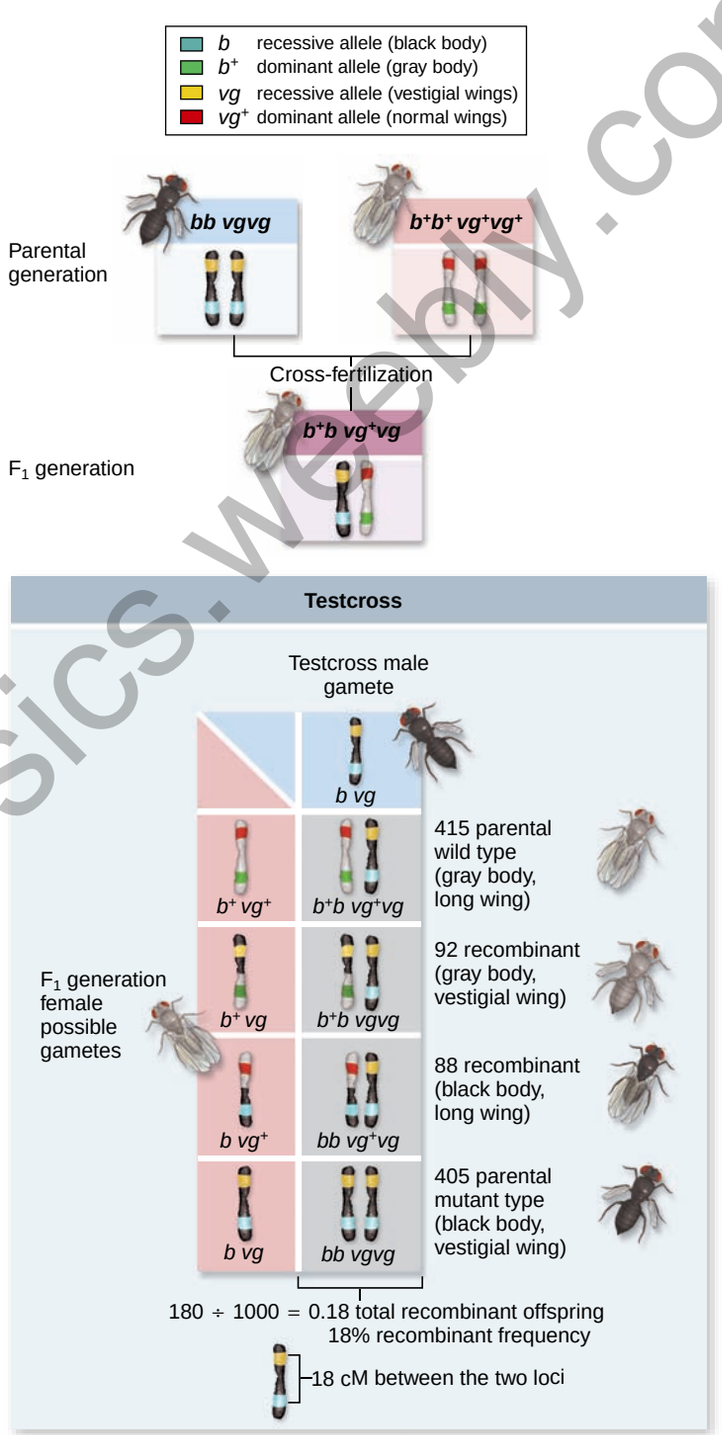


Figure 13.7 Two-point cross to map genes. Flies homozygous for long wings *(vg^+)* and gray bodies *(b^+)* are crossed to flies homozygous for vestigial wings *(vg)* and black bodies *(b)*. Both vestigial wing and black body are recessive to the normal (wild-type) long wing and grey body. The F_1 progeny are then testcrossed to homozygous vestigial black to produce the progeny for mapping. Data are analyzed in the text.

heterozygous F_1 progeny are then testcrossed to homozygous recessive individuals *(vg b/vg b)*, and progeny are counted (figure 13.7). The data are shown here:

vestigial wings, black body *(vg b)*	405	(parental)
long wings, gray body *(vg^+ b^+)*	415	(parental)
vestigial wings, gray body *(vg b^+)*	92	(recombinant)
long wings, black body *(vg^+ b)*	88	(recombinant)
Total Progeny	1000	

The numbers of recombinant progeny are added together, and this sum is divided by total progeny to produce the recombination frequency. The recombination frequency is 92 + 88 divided by 1000, or 0.18. Converting this number to a percentage yields 18 cM as the map distance between these two loci.

Multiple crossovers can yield independent assortment results

As the distance separating loci increases, the probability of recombination occurring between them during meiosis also increases. What happens when more than one recombination event occurs?

If homologues undergo two crossovers between loci, then the parental combination is restored. This leads to an underestimate of the true genetic distance because not all events can be noted. As a result, the relationship between true distance on a chromosome and the recombination frequency is not linear. It begins as a straight line, but the slope decreases; the curve levels off at a recombination frequency of 0.5 (figure 13.8).

At long distances, multiple events between loci become frequent. In this case, odd numbers of crossovers (1, 3, 5) produce recombinant gametes, and no crossover or even numbers of crossovers (0, 2, 4) produce parental gametes. At large enough distances, these frequencies are about equal, leading to the number of recombinant gametes being equal to the number of parental gametes, and the loci exhibit independent assortment! This is how Mendel could use two loci on the same chromosome and have them assort independently.

Inquiry question

What would Mendel have observed in a dihybrid cross if the two loci were 10 cM apart on the same chromosome? Is this likely to have led him to the idea of independent assortment?

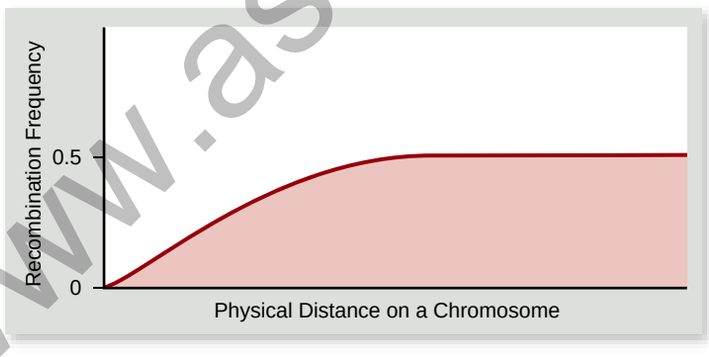


Figure 13.8 Relationship between true distance and recombination frequency. As distance on a chromosome increases, the recombinants are not all detected due to double crossovers. This leads to a curve that levels off at 0.5.

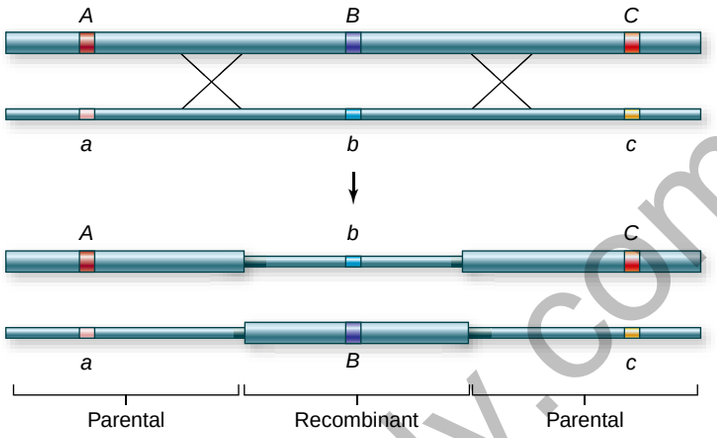


Figure 13.9 Use of a three-point cross to order genes. In a two-point cross, the outside loci appear parental for double crossovers. With the addition of a third locus, the two crossovers can still be detected because the middle locus will be recombinant. This double crossover class should be the least frequent, so whatever locus has recombinant alleles in this class must be in the middle.

Three-point crosses can be used to put genes in order

Because multiple crossovers reduce the number of observed recombinant progeny, longer map distances are not accurate. As a result, when geneticists try to construct maps from a series of two-point crosses, determining the order of genes is problematic. Using three loci instead of two, or a three-point cross, can help solve the problem.

In a three-point cross, the gene in the middle allows us to see recombination events on either side. For example, a double crossover for the two outside loci is actually a single crossover between the middle locus and each outside locus (figure 13.9).

The probability of two crossovers is equal to the product of the probability of each individual crossover, each of which is relatively low. Therefore, in any three-point cross, the class of offspring with two crossovers is the least frequent class. Analyzing these individuals to see which locus is recombinant identifies the locus that lies in the middle of the three loci in the cross (see figure 13.9).

In practice, geneticists use three-point crosses to determine the order of genes, then use data from the closest two-point crosses to determine distances. Longer distances are generated by simple addition of shorter distances. This avoids using inaccurate measures from two-point crosses between distant loci.

Genetic maps can be constructed for the human genome

Human genes can be mapped, but the data must be derived from historical pedigrees, such as those of the royal families of Europe mentioned earlier. The principle is the same—genetic distance is still proportional to recombination frequency—but the analysis requires the use of complex statistics and summing data from many families.

The difficulty of mapping in humans

Looking at nonhuman animals with extensive genetic maps, the majority of genetic markers have been found at loci where alleles

cause morphological changes, such as variant eye color, body color, or wing morphology in flies. In humans, such alleles generally, but not always, correspond to what we consider disease states. As recently as the early 1980s, the number of markers for the human genome numbered in the hundreds. Because the human genome is so large, however, this low number of markers would never provide dense enough coverage to use for mapping.

Another consideration is that the disease-causing alleles are those that we wish to map, but they occur at low frequencies in the population. Any one family would be highly unlikely to carry multiple disease alleles, the segregation of which would allow for mapping.

Anonymous markers

This situation changed with the development of **anonymous markers,** genetic markers that can be detected using molecular techniques, but that do not cause a detectable phenotype. The nature of these markers has evolved with technology, leading to a standardized set of markers scattered throughout the genome. These markers, which have a relatively high density, can be detected using techniques that are easy to automate. As a result of analysis, geneticists now have several thousand markers to work with, instead of hundreds, and have produced a human genetic map that would have been unthinkable 25 years ago (figure 13.10). (In the following chapters of this unit, you'll learn about some of the molecular techniques that have been developed for use with genomes.)

Single-nucleotide polymorphisms (SNPs)

The information developed from sequencing the human genome can then be used to identify and map single bases that differ between individuals. Any differences between individuals in populations are termed *polymorphisms;* polymorphisms affecting a single base of a gene locus are called **single-nucleotide polymorphisms (SNPs).** Over 2 million such differences have been identified and are being placed on both the genetic map and the human genome sequence. This confluence of techniques will enable the ultimate resolution of genetic analysis.

The recent progress in gene mapping applies to more than just the relatively small number of genes that show simple Mendelian inheritance. The development of a high-resolution genetic map, and the characterization of millions of SNPs, opens up the possibility of being able to characterize complex quantitative traits in humans as well.

On a more practical level, the types of molecular markers described earlier are used in forensic analysis. Although not quite as rapid as some television programs would have you believe, this does allow rapid DNA testing of crime scene samples to help eliminate or confirm crime suspects and for paternity testing.

Learning Outcomes Review 13.4

Crossing over during meiosis exchanges alleles on homologues. This recombination of alleles can be used to map the location of genes. Genes that are close together are said to be linked, and exhibit an excess of parental versus recombinant types in a testcross. The frequency of recombination in testcrosses is used as a measure of genetic distance. Loci separated by large distances have multiple crossovers between them, which can lead to independent assortment.

- ***If two genes assort independently, can you tell if they are on a single chromosome and far apart or on two different chromosomes?***

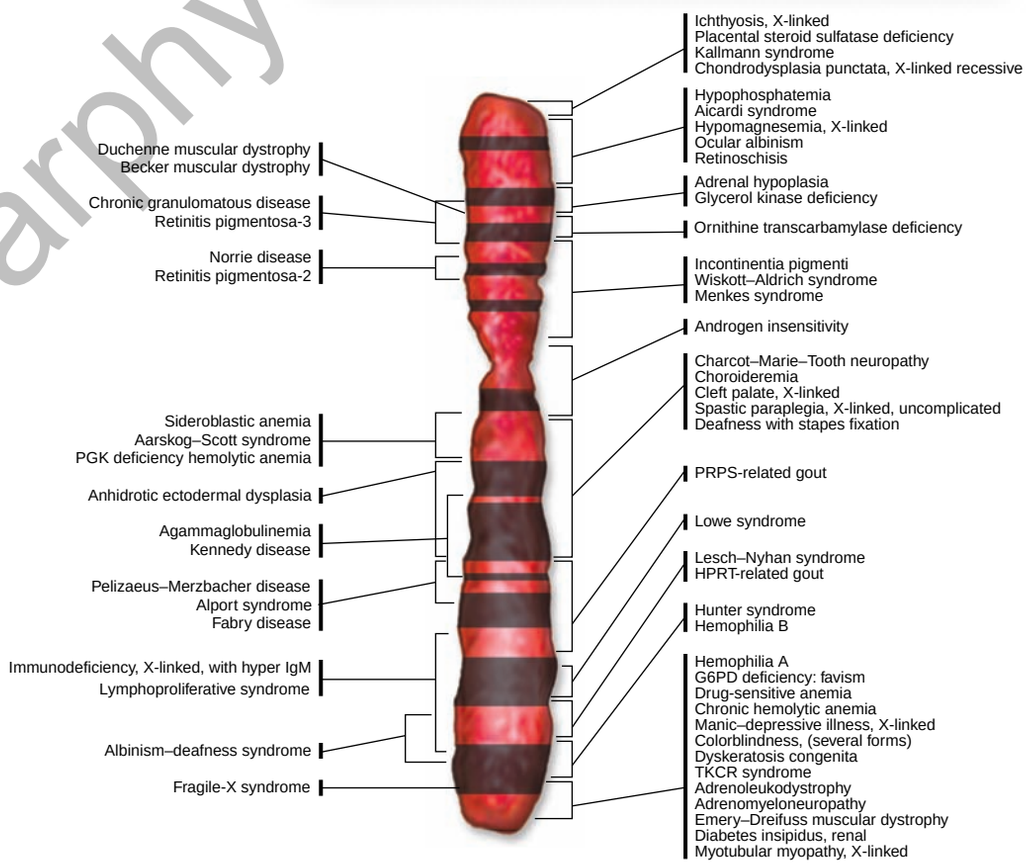


Figure 13.10 The human X chromosome gene map. Only a partial map for the human X chromosome is presented here, a more detailed map would require a much larger figure. The black bands represent staining patterns that can be seen under the microscope, and the constriction represents the centromere. Analysis of the sequence of the X chromosome indicates 1098 genes on the X chromosome. Many of these may have mutant alleles that can affect disease states. By analyzing inheritance patterns of affected and unaffected individuals, the 59 diseases shown have been traced to specific segments of the X chromosome, indicated by brackets.

13.5 Selected Human Genetic Disorders

Learning Outcomes

1. *Explain how mutations can cause disease.*
2. *Describe the consequences of nondisjunction in humans.*
3. *Recognize how genomic imprinting can lead to non-Mendelian inheritance.*

Diseases that run in families have been known for many years. These can be nonlife-threatening like albinism, or may result in premature death like Huntington's, which were used as examples of recessive and dominant traits in humans previously. A small sample of diseases due to alterations of alleles of a single gene is provided in table 13.2. We will discuss the nature of these genetic changes later in chapter 15. In this section we discuss some of the genetic disorders that have been found in human populations.

Sickle cell anemia is due to altered hemoglobin

The first human disease shown to be the result of a mutation in a protein was sickle cell anemia. It is caused by a defect in the oxygen carrier molecule, hemoglobin, that leads to impaired oxygen delivery to tissues. The defective hemoglobin molecules stick to one another, leading to stiff, rodlike structures that alter the shape of the red blood cells that carry them. These red blood cells take on a characteristic shape that led to the name "sickle cell" (figure 13.11).

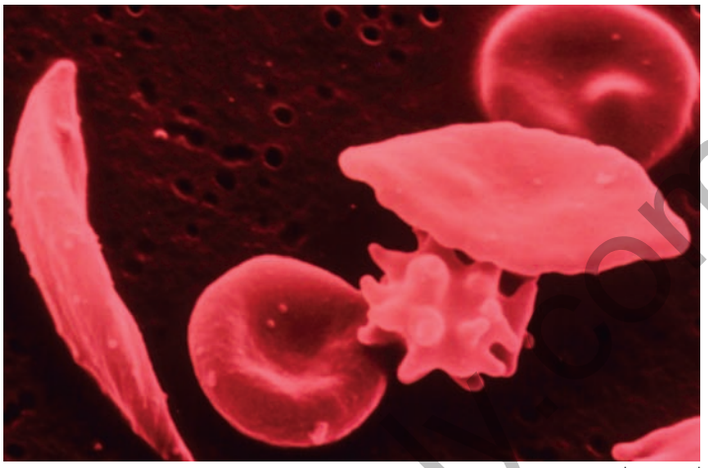


Figure 13.11 Sickle cell anemia. In individuals homozygous for the sickle cell trait, many of the red blood cells have sickled or irregular shapes, such as the cell on the far left.

Individuals homozygous for the sickle cell allele exhibit intermittent illness and reduced life span. Individuals heterozygous for the sickle cell allele are indistinguishable from normal individuals in a normal oxygen environment, although their red cells do exhibit reduced ability to carry oxygen.

The sickle cell allele is particularly prevalent in people of African descent. In some regions of Africa, up to 45% of the population is heterozygous for the trait, and 6% are homozygous. This proportion of heterozygotes is higher than would be expected on the basis of chance alone. It turns out that heterozygosity confers a greater resistance to the blood-borne parasite

TABLE 13.2 Some Important Genetic Disorders

Disorder	Symptom	Defect	Dominant/Recessive	Frequency Among Human Births
Cystic fibrosis	Mucus clogs lungs, liver, and pancreas	Failure of chloride ion transport mechanism	Recessive	1/2500 (Caucasians)
Sickle cell anemia	Blood circulation is poor	Abnormal hemoglobin molecules	Recessive	1/600 (African Americans)
Tay–Sachs disease	Central nervous system deteriorates in infancy	Defective enzyme (hexosaminidase A)	Recessive	1/3500 (Ashkenazi Jews)
Phenylketonuria	Brain fails to develop in infancy, treatable with dietary restriction	Defective enzyme (phenylalanine hydroxylase)	Recessive	1/12,000
Hemophilia	Blood fails to clot	Defective blood-clotting factor VIII	X-linked recessive	1/10,000 (Caucasian males)
Huntington disease	Brain tissue gradually deteriorates in middle age	Production of an inhibitor of brain cell metabolism	Dominant	1/24,000
Muscular dystrophy (Duchenne)	Muscles waste away	Degradation of myelin coating of nerves stimulating muscles	X-linked recessive	1/3700 (males)
Hypercholesterolemia	Excessive cholesterol levels in blood lead to heart disease	Abnormal form of cholesterol cell surface receptor	Dominant	1/500

that causes malaria. In regions of central Africa where malaria is endemic, the sickle cell allele also occurs at a high frequency.

The sickle cell allele is not the end of the story for the β-globin gene; a large number of other alterations of this gene have been observed that lead to anemias. In fact, for hemoglobin, which is composed of two α-globins and two β-globins, over 700 structural variants have been cataloged. It is estimated that 7% of the human population worldwide are carriers for different inherited hemoglobin disorders.

The Human Gene Mutation Database has cataloged the nature of many disease alleles, including the sickle cell allele. The majority of alleles seem to be simple changes. Almost 60% of the close to 28,000 alleles in the Human Gene Mutation Database are single-base substitutions. Another 23% are due to small insertions or deletions of less than 20 bases. The rest of the alleles are made of more complex alterations. It is clear that simple changes in genes can have profound effects.

Nondisjunction of chromosomes changes chromosome number

The failure of homologues or sister chromatids to separate properly during meiosis is called **nondisjunction.** This failure leads to the gain or loss of a chromosome, a condition called **aneuploidy.** The frequency of aneuploidy in humans is surprisingly high, being estimated to occur in 5% of conceptions.

Nondisjunction of autosomes

Humans who have lost even one copy of an autosome are called **monosomics,** and generally do not survive embryonic development. In all but a few cases, humans who have gained an extra autosome (called **trisomics**) also do not survive. Data from clinically recognized spontaneous abortions indicate levels of aneuploidy as high as 35%.

Five of the smallest human autosomes—those numbered 13, 15, 18, 21, and 22—can be present as three copies and still allow the individual to survive, at least for a time. The presence of an extra chromosome 13, 15, or 18 causes severe developmental defects, and infants with such a genetic makeup die within a few months. In contrast, individuals who have an extra copy of chromosome 21 or, more rarely, chromosome 22, usually survive to adulthood. In these people, the maturation of the skeletal system is delayed, so they generally are short and have poor muscle tone. Their mental development is also affected, and children with trisomy 21 are always mentally retarded to some degree.

The developmental defect produced by trisomy 21 (figure 13.12) was first described in 1866 by J. Langdon Down; for this reason, it is called Down syndrome. About 1 in every 750 children exhibits Down syndrome, and the frequency is comparable in all racial groups. Similar conditions also occur in chimpanzees and other related primates.

In humans, the defect occurs when a particular small portion of chromosome 21 is present in three copies instead of two. In 97% of the cases examined, all of chromosome 21 is present in three copies. In the other 3%, a small portion of chromosome 21 containing the critical segment has been added to another chromosome by a process called *translocation* (see chapter 15);

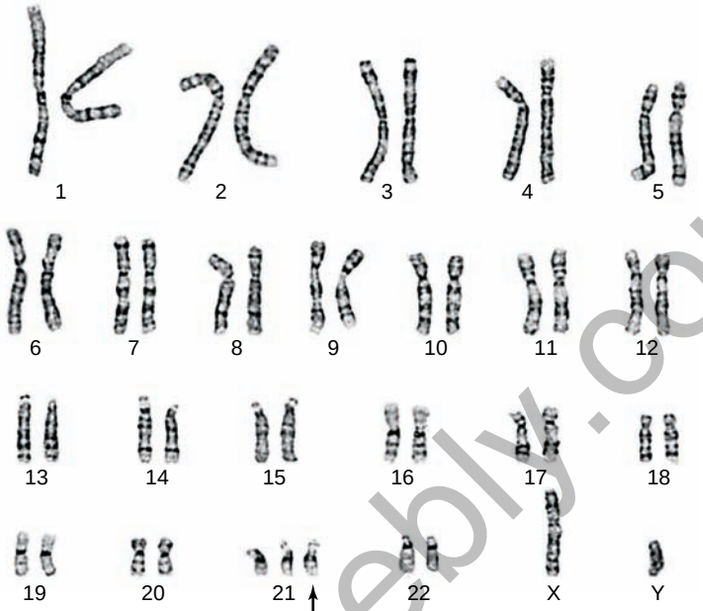


Figure 13.12 Down syndrome. As shown in this male karyotype, Down syndrome is associated with trisomy of chromosome 21 (arrow shows third copy of chromosome 21).

it exists along with the normal two copies of chromosome 21. This latter condition is known as *translocation Down syndrome.*

In mothers younger than 20 years of age, the risk of giving birth to a child with Down syndrome is about 1 in 1700; in mothers 20 to 30 years old, the risk is only about 1 in 1400. However, in mothers 30 to 35 years old, the risk rises to 1 in 750, and by age 45, the risk is as high as 1 in 16 (figure 13.13).

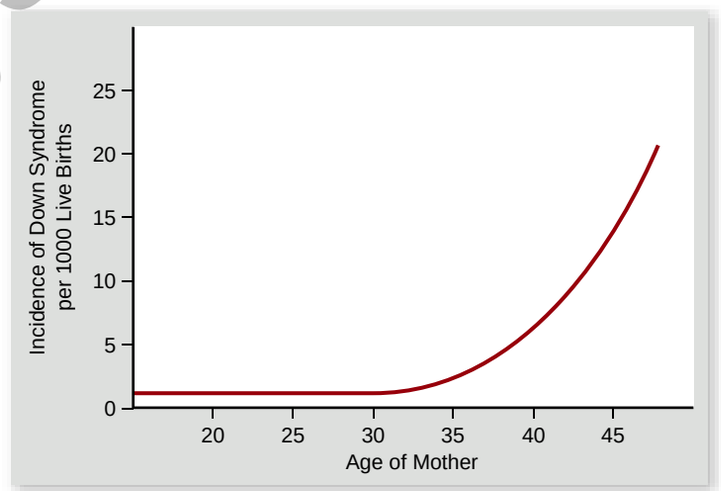


Figure 13.13 Correlation between maternal age and the incidence of Down syndrome. As women age, the chances they will bear a child with Down syndrome increase. After a woman reaches 35, the frequency of Down syndrome rises rapidly.

Inquiry question

Over a five-year period between ages 20 and 25, the incidence of Down syndrome increases 0.1 per thousand; over a five-year period between ages 35 and 40, the incidence increases to 8.0 per thousand, 80 times as great. The period of time is the same in both instances. What has changed?

Primary nondisjunctions are far more common in women than in men because all of the eggs a woman will ever produce have developed to the point of prophase in meiosis I by the time she is born. By the time a woman has children, her eggs are as old as she is. Therefore, there is a much greater chance for cell-division problems of various kinds, including those that cause primary nondisjunction, to accumulate over time in female gametes. In contrast, men produce new sperm daily. For this reason, the age of the mother is more critical than that of the father for couples contemplating childbearing.

Nondisjunction of sex chromosomes

Individuals who gain or lose a sex chromosome do not generally experience the severe developmental abnormalities caused by similar changes in autosomes. Although such individuals have somewhat abnormal features, they often reach maturity and in some cases may be fertile.

X chromosome nondisjunction. When X chromosomes fail to separate during meiosis, some of the gametes produced possess both X chromosomes, and so are XX gametes; the other gametes have no sex chromosome and are designated "O" (figure 13.14).

If an XX gamete combines with an X gamete, the resulting XXX zygote develops into a female with one functional X chromosome and two Barr bodies. She may be taller in stature but is otherwise normal in appearance.

If an XX gamete instead combines with a Y gamete, the effects are more serious. The resulting XXY zygote develops into a male who has many female body characteristics and, in some cases but not all, diminished mental capacity. This condition, called *Klinefelter syndrome*, occurs in about 1 out of every 500 male births.

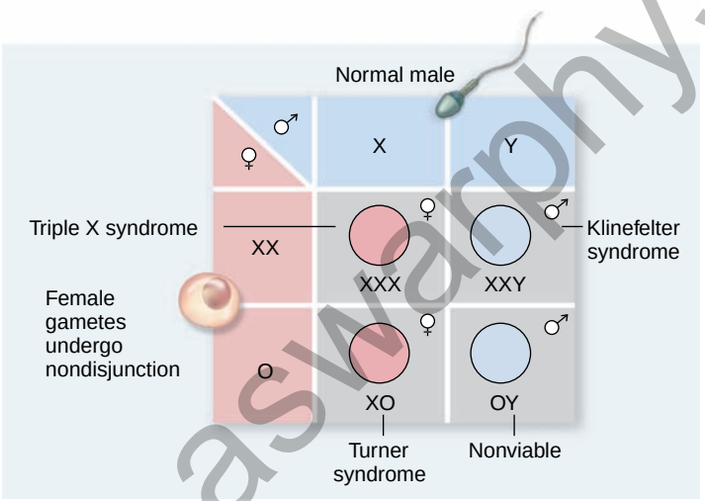


Figure 13.14 How nondisjunction can produce abnormalities in the number of sex chromosomes. When nondisjunction occurs in the production of female gametes, the gamete with two X chromosomes (XX) produces Klinefelter males (XXY) and triple-X females (XXX). The gamete with no X chromosome (O) produces Turner females (XO) and nonviable OY males lacking any X chromosome.

Inquiry question

Can you think of two nondisjunction scenarios that would produce an XXY male?

If an O gamete fuses with a Y gamete, the resulting OY zygote is nonviable and fails to develop further; humans cannot survive when they lack the genes on the X chromosome. But if an O gamete fuses with an X gamete, the XO zygote develops into a sterile female of short stature, with a webbed neck and sex organs that never fully mature during puberty. The mental abilities of an XO individual are in the low-normal range. This condition, called *Turner syndrome*, occurs roughly once in every 5000 female births.

Y chromosome nondisjunction. The Y chromosome can also fail to separate in meiosis, leading to the formation of YY gametes. When these gametes combine with X gametes, the XYY zygotes develop into fertile males of normal appearance. The frequency of the XYY genotype *(Jacob syndrome)* is about 1 per 1000 newborn males.

Genomic imprinting depends on the parental origin of alleles

By the late 20th century, geneticists were confident that they understood the basic mechanisms governing inheritance. It came as quite a surprise when mouse geneticists found an important exception to classical Mendelian genetics that appears to be unique to mammals. In **genomic imprinting,** the phenotype caused by a specific allele is exhibited when the allele comes from one parent, but not from the other.

The basis for genomic imprinting is the expression of a gene depending on passage through maternal or paternal germ lines. Some genes are inactivated in the paternal germ line and therefore are not expressed in the zygote. Other genes are inactivated in the maternal germ line, with the same result. This condition makes the zygote effectively haploid for an imprinted gene. The expression of variant alleles of imprinted genes depends on the parent of origin. Furthermore, imprinted genes seem to be concentrated in particular regions of the genome. These regions include genes that are both maternally and paternally imprinted.

Prader–Willi and Angelman syndromes

An example of genomic imprinting in humans involves the two diseases Prader–Willi syndrome (PWS) and Angelman syndrome (AS). The effects of PWS include respiratory distress, obesity, short stature, mild mental retardation, and obsessive–compulsive behavior. The effects of AS include developmental delay, severe mental retardation, hyperactivity, aggressive behavior, and inappropriate laughter.

Genetic studies have implicated genes on chromosome 15 for both disorders, but the pattern of inheritance is complementary. The most common cause of both syndromes is a deletion of material on chromosome 15 and, in fact, the same deletion can cause either syndrome. The determining factor is the parental origin of the normal and deleted chromosomes. If the chromosome with the deletion is paternally inherited it causes PWS, if the chromosome with the deletion is maternally inherited it causes AS.

The region of chromosome 15 that is lost is subject to imprinting, with some genes being inactivated in the maternal germ line, and others in the paternal germ line. In PWS, genes

are inactivated in the maternal germ line, such that deletion or other functional loss of paternally derived alleles produces the syndrome. The opposite is true for AS syndrome: Genes are inactivated in the paternal germ line, such that loss of maternally derived alleles leads to the syndrome.

Molecular basis of genomic imprinting

Although genomic imprinting is not well understood, at least one aspect seems clear: The basis for inactivating genes appears to be linked to modifications of the DNA itself. DNA can be modified by the addition of methyl groups, termed *methylation*. This modification is correlated with inactivity of genes. The proteins that are associated with chromosomes can also be modified, leading to effects on gene expression. The control of gene expression is discussed in more detail in the following chapters.

Some genetic defects can be detected early in pregnancy

Although most genetic disorders cannot yet be cured, we are learning a great deal about them, and progress toward successful therapy is being made in many cases. In the absence of a cure, however, the only recourse is to try to avoid producing children with these conditions. The process of identifying parents at risk for having children with genetic defects and of assessing the genetic state of early embryos is called **genetic counseling.**

Pedigree analysis

One way of assessing risks is through pedigree analysis, often employed as an aid in genetic counseling. By analyzing a person's pedigree, it is sometimes possible to estimate the likelihood that the person is a carrier for certain disorders. For example, if a counseling client's family history reveals that a relative has been afflicted with a recessive genetic disorder, such as cystic fibrosis, it is possible that the client is a heterozygous carrier of the recessive allele for that disorder.

When a couple is expecting a child, and pedigree analysis indicates that both of them have a significant chance of being heterozygous carriers of a deleterious recessive allele, the pregnancy is said to be high-risk. In such cases, a significant probability exists that their child will exhibit the clinical disorder.

Another class of high-risk pregnancy is that in which the mothers are older than 35. As discussed earlier, the frequency of Down syndrome increases dramatically in the pregnancies of older women (see figure 13.13).

Amniocentesis

When a pregnancy is diagnosed as high-risk, many women elect to undergo **amniocentesis,** a procedure that permits the prenatal diagnosis of many genetic disorders. In the fourth month of pregnancy, a sterile hypodermic needle is inserted into the expanded uterus of the mother, removing a small sample of the amniotic fluid that bathes the fetus (figure 13.15). Within the fluid are free-floating cells derived from the fetus; once removed, these cells can be grown in cultures in the laboratory.

During amniocentesis, the position of the needle and that of the fetus are usually observed by means of *ultrasound.* The sound waves used in ultrasound are not harmful to mother or fetus, and they permit the person withdrawing the amniotic fluid to do so without damaging the fetus. In addition, ultrasound can be used to examine the fetus for signs of major abnormalities. However, about 1 out of 200 amniocentesis procedures may result in fetal death and miscarriage.

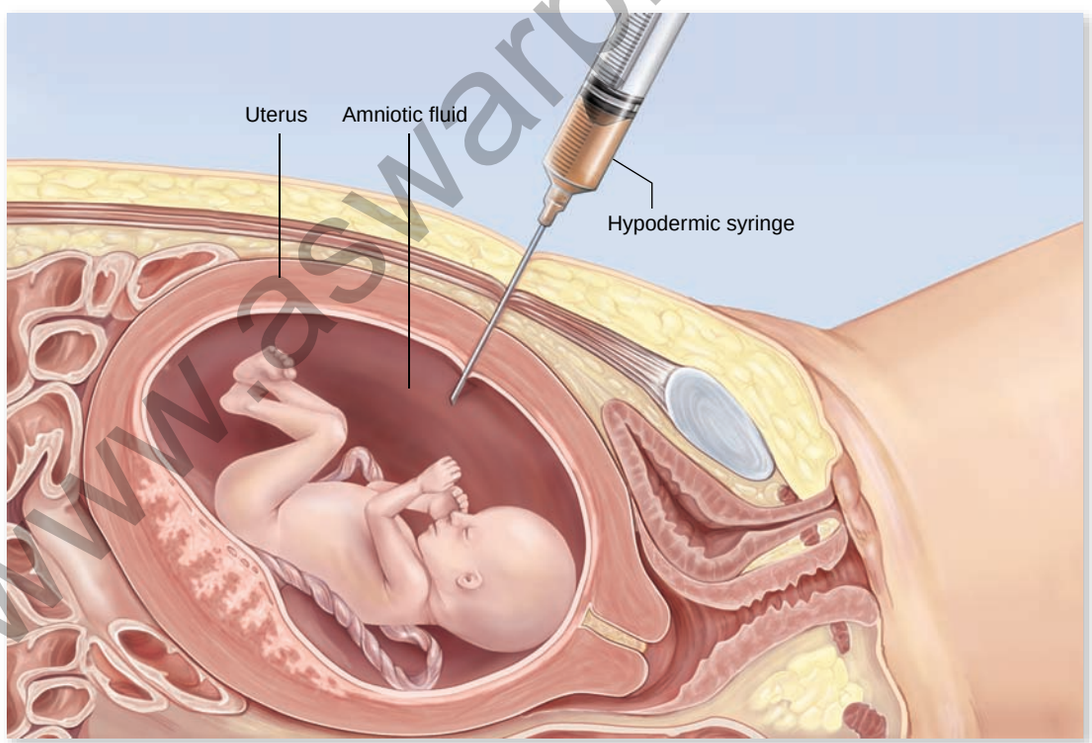


Figure 13.15
Amniocentesis. A needle is inserted into the amniotic cavity, and a sample of amniotic fluid, containing some free cells derived from the fetus, is withdrawn into a syringe. The fetal cells are then grown in culture, and their karyotype and many of their metabolic functions are examined.

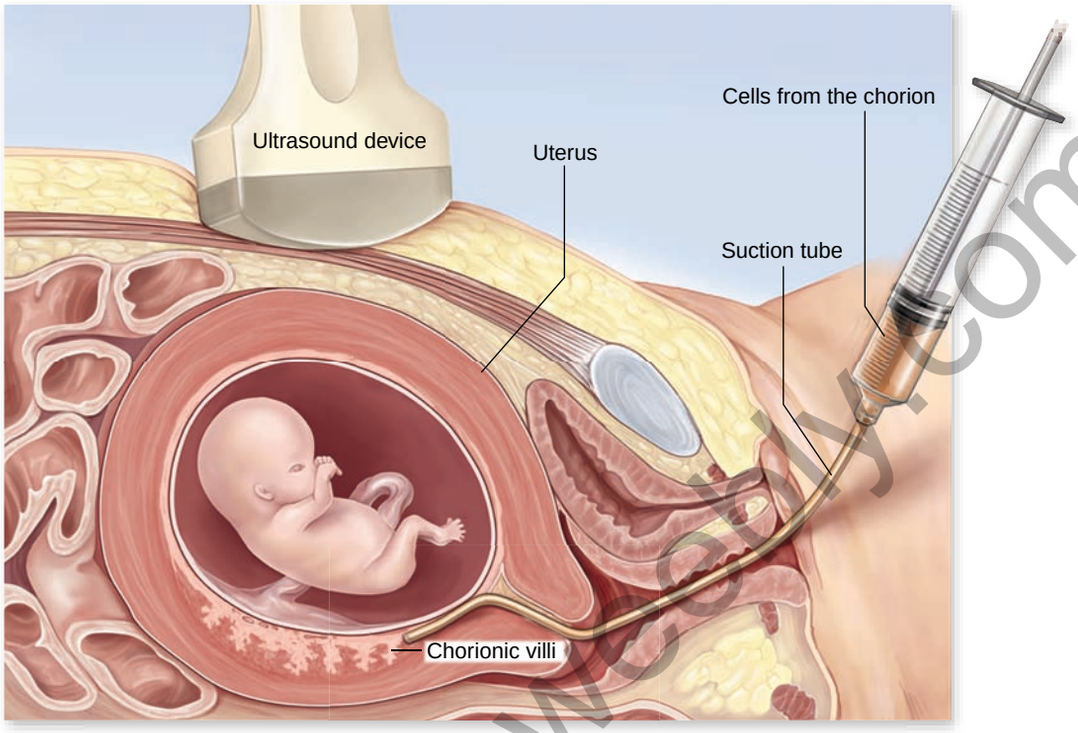


Figure 13.16 Chorionic villi sampling. Cells can be taken from the chorionic villi as early as the eighth to tenth week of pregnancy. Cells are removed by suction with a tube inserted through the cervix. These cells can then be grown in culture and examined for karyotypes and tested biochemically for defects.

Chorionic villi sampling

In recent years, physicians have increasingly turned to a new, less invasive procedure for genetic screening called **chorionic villi sampling.** Using this method, the physician removes cells from the chorion, a membranous part of the placenta that nourishes the fetus (figure 13.16). This procedure can be used earlier in pregnancy (by the eighth week) and yields results much more rapidly than does amniocentesis. Risks from chorionic villi sampling are comparable to those for amniocentesis.

To test for certain genetic disorders, genetic counselors look for three characteristics in the cultures of cells obtained from amniocentesis or chorionic villi sampling. First, analysis of the karyotype can reveal aneuploidy (extra or missing chromosomes) and gross chromosomal alterations. Second, in many cases it is possible to test directly for the proper functioning of enzymes involved in genetic disorders. The lack of normal enzymatic activity signals the presence of the disorder. As examples, the lack of the enzyme responsible for breaking down phenylalanine indicates phenylketonuria (PKU); the absence of the enzyme responsible for the breakdown of gangliosides indicates Tay–Sachs disease; and so forth. Additionally, with information from the Human Genome Project, more disease alleles for genetic disorders are known. If there are a small number of alleles for a specific disease in the population, these can be identified as well.

With the changes in human genetics brought about by the Human Genome Project (see chapter 18), it is possible to design tests for many more diseases. Difficulties still exist in discerning the number and frequency of disease-causing alleles, but these problems are not insurmountable. At present, tests for at least 13 genes with alleles that lead to clinical syndromes are available. This number is bound to rise and to be expanded to include alleles that do not directly lead to disease states but that predispose a person for a particular disease.

Inquiry question

Based on what you read in this chapter, what reasons could a mother have to undergo CVS, considering its small but potential risks?

Learning Outcomes Review 13.5

Mutations in DNA that result in altered proteins can cause hereditary diseases. Pedigree studies and genetic testing may clarify the risk of disease. At the chromosome level, nondisjunction during meiosis can result in gametes with too few or too many chromosomes, most of which produce inviable offspring. Imprinting refers to inactivation of alleles depending on which parent the alleles come from; offspring in whom imprinting occurs appear haploid for the affected gene even though they are diploid.

- ***During spermatogenesis, is there any difference in outcome between first and second division nondisjunction?***

Chapter Review

13.1 Sex Linkage and the Chromosomal Theory of Inheritance

Morgan correlated the inheritance of a trait with sex chromosomes (see figure 13.2).

Morgan crossed red-eyed and white-eyed flies and found differences in inheritance based on the sex of offspring. All white-eyed offspring were males, but testcrosses showed that white-eyed females were possible, supporting the idea that the white-eye gene was on the X chromsome.

The gene for eye color lies on the X chromosome.

The inheritance of eye color in *Drosophila* segregates with the X chromosome, a phenomenon termed sex-linked inheritance.

13.2 Sex Chromosomes and Sex Determination

Sex determination in animals is usually associated with a chromosomal difference. In some animals, females have two similar sex chromosomes and males have sex chromosomes that differ. In other species, females have sex chromosomes that differ (see table 13.1).

In humans the Y chromosome generally determines maleness.

The Y chromosome is highly condensed and does not have active counterparts to most genes on the X chromosome. The *SRY* gene on the Y chromosome is responsible for the masculinization of genitalia and secondary sex organs. An XY individual can develop into a sterile female due to mutations in the *SRY* gene or the failure of the embryo to respond to androgens.

Some human genetic disorders display sex linkage (see figure 13.3).

Human genetic disorders show sex linkage when the relevant gene is on the X chromosome; hemophilia is an example.

Dosage compensation prevents doubling of sex-linked gene products.

In fruit flies, males double the gene expression from their single X chromosome. In mammals, one of the X chromosomes in a female is randomly inactivated during development.

X-chromosome inactivation can lead to genetic mosaics.

In a mammalian female that is heterozygous for X-chromosome alleles, X inactivation produces a mosaic pattern, as shown in the coat color of calico cats (see figure 13.4).

13.3 Exceptions to the Chromosomal Theory of Inheritance

Mitochondrial genes are inherited from the female parent.

Mitochondria have their own genomes and divide independently; they are passed to offspring in the cytoplasm of the egg cell.

Chloroplast genes may also be passed on uniparentally.

Chloroplasts also reside in the cytoplasm, have their own genomes, and divide independently. They are usually inherited maternally.

13.4 Genetic Mapping

Mendel's independent assortment is too simplistic. Genes on the same chromosome may or may not segregate independently.

Genetic recombination exchanges alleles on homologues.

Homologous chromosomes may exchange alleles by crossing over (see figure 13.5). This occurs by breakage and rejoining of chromosomes as shown by crosses in which chromosomes carry both visible and genetic markers (see figure 13.6).

Recombination is the basis for genetic maps.

Genes close together on a single chromosome are said to be linked. The further apart two linked genes are, the greater the frequency of recombination. This allows genetic maps to be constructed based on recombination frequency. A map unit is expressed as the percentage of recombinant progeny.

Multiple crossovers can yield independent assortment results.

The probability of multiple crossovers increases with distance between two genes and results in an underestimate of recombination frequency. The maximum recombination frequency is 50%, the same value as for independent assortment.

Three-point crosses can be used to put genes in order (see figure 13.9).

If three genes are used instead of two, data from multiple crossovers can be used to order genes. Longer map distances fail to reflect the effect of multiple crossovers and thus underestimate true distance. By evaluating intervening genes with less separation, more accurate distances can be obtained.

Genetic maps can be constructed for the human genome.

Human genetic mapping was difficult because it required multiple disease-causing alleles segregating in a family. The process has been made easier by the use of anonymous markers, identifiable molecular markers that do not cause a phenotype. Single-nucleotide polymorphisms (SNPs) can be used to detect differences between individuals for identification.

13.5 Selected Human Genetic Disorders

Sickle cell anemia is due to altered hemoglobin.

The phenotypes in sickle cell anemia can all be traced to alterations in the structure of hemoglobin that affect the shape of red blood cells. Over 700 variants of hemoglobin structure have been characterized, some of which also cause disorders.

Nondisjunction of chromosomes changes chromosome number.

Nondisjunction is the failure of homologues or sister chromatids to separate during meiosis. The result is aneuploidy: monosomy or trisomy of a chromosome in the zygote. Most aneuploidies are lethal, but some, such as trisomy 21 in humans (Down syndrome), can result in viable offspring. X-chromosome nondisjunction occurs when X chromosomes fail to separate during meiosis. The resulting gamete carries either XX or O (zero sex chromosomes) (see figure 13.14). Y-chromosome nondisjunction results in YY gametes.

Genomic imprinting depends on the parental origin of alleles.

In genomic imprinting, the expression of a gene depends on whether it passes through the maternal or paternal germ line. Imprinted genes appear to be inactivated by methylation. Imprinting produces a haploid phenotype.

Some genetic defects can be detected early in pregnancy.

Genetic defects in humans can be determined by pedigree analysis, amniocentesis, or chorionic villi sampling.

Review Questions

UNDERSTAND

1. Why is the white-eye phenotype always observed in males carrying the white-eye allele?
   a. Because the trait is dominant
   b. Because the trait is recessive
   c. Because the allele is located on the X chromosome and males only have one X
   d. Because the allele is located on the Y chromosome and only males have Y chromosomes
2. In an organism's genome, *autosomes* are
   a. the chromosomes that differ between the sexes.
   b. chromosomes that are involved in sex determination.
   c. only inherited from the mother (maternal inheritance).
   d. all of the chromosomes other than sex chromosomes.
3. What cellular process is responsible for genetic recombination?
   a. The independent alignment of homologous pairs during meiosis I
   b. Separation of the homologues in meiosis I
   c. Separation of the chromatids during meiosis II
   d. Crossing over between homologues
4. The map distance between two genes is determined by the
   a. recombination frequency.
   b. frequency of parental types.
   c. ratio of genes to length of a chromosome.
   d. ratio of parental to recombinant progeny.
5. How many map units separate two alleles if the recombination frequency is 0.07?
   a. 700 cM b. 70 cM c. 7 cM d. 0.7 cM
6. How does maternal inheritance of mitochondrial genes differ from sex linkage?
   a. Mitochondrial genes do not contribute to the phenotype of an individual.
   b. Because mitochondria are inherited from the mother, only females are affected.
   c. Since mitochondria are inherited from the mother, females and males are equally affected.
   d. Mitochondrial genes must be dominant. Sex-linked traits are typically recessive.
7. Which of the following genotypes due to nondisjunction of sex chromosomes is lethal?
   a. XXX b. XXY c. OY d. XO

APPLY

1. Dosage compensation is needed to
   a. balance expression from autosomes relative to sex chromosomes.
   b. balance expression from two autosomes in a diploid cell.
   c. balance expression of sex chromosomes in both sexes.
   d. inactivate female-specific autosomal chromosomes.
2. As real genetic distances increases, the distance calculated by recombination frequency becomes an
   a. overestimate due to multiple crossovers that cannot be scored.
   b. underestimate due to multiple crossovers that cannot be scored.
   c. underestimate due to multiple crossovers adding to recombination frequency.
   d. overestimate due to multiple crossovers adding to recombination frequency.
3. Down syndrome is the result of trisomy for chromosome 21. Why is this trisomy viable and trisomy for most other chromosomes is not?
   a. Chromosome 21 is a large chromosome and excess genetic material is less harmful.
   b. Chromosome 21 behaves differently in meiosis I than the other chromosomes.
   c. Chromosome 21 is a small chromosome with few genes so this does less to disrupt the genome.
   d. Chromosome 21 is less prone to nondisjunction than other chromosomes.
4. Genes that are on the same chromosome can show independent assortment
   a. when they are far enough apart for two crossovers to occur.
   b. when they are far enough apart that odd numbers of crossovers is about equal to even.
   c. only if recombination is low for that chromosome.
   d. only if the genes show genomic imprinting.
5. We use three-point crosses to order genes because this allows us to
   a. control for dosage compensation.
   b. control for genomic imprinting.
   c. detect multiple recombination events, and these infrequent events give us the order.
   d. detect multiple recombination events, and these frequent events give us the order.
6. During the process of spermatogenesis, a nondisjunction event that occurs during the second division would be
   a. worse than the first division because all four meiotic products would be aneuploid.
   b. better than the first division because only two of the four meiotic products would be aneuploid.
   c. the same outcome as the first division with all four products aneuploid.
   d. the same outcome as the first division as only two products would be aneuploid.

SYNTHESIZE

1. Color blindness is caused by a sex-linked, recessive gene. If a woman, whose father was color blind, marries a man with normal color vision, what percentage of their children will be color blind? What percentage of male children? Of female children?
2. Assume that the genes for seed color and seed shape are located on the same chromosome. A plant heterozygous for both genes is testcrossed wrinkled green with the following results:

green, wrinkled	645
green, round	36
yellow, wrinkled	29
yellow, round	590

   What were the genotypes of the parents, and how far apart are these genes?
3. Is it possible to have a calico cat that is male? Why or why not?

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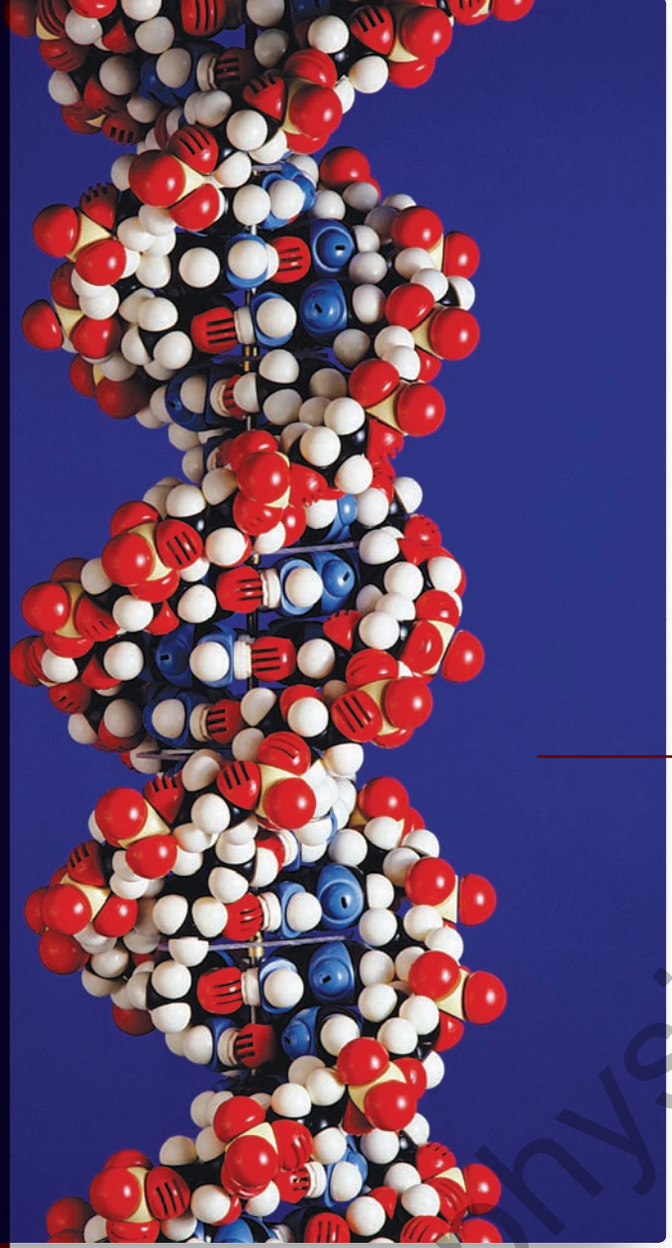


Chapter 14

DNA: The Genetic Material

Chapter Outline

Introduction

The realization that patterns of heredity can be explained by the segregation of chromosomes in meiosis raised a question that occupied biologists for over 50 years: What is the exact nature of the connection between hereditary traits and chromosomes? This chapter describes the chain of experiments that led to our current understanding of DNA, modeled in the picture, and of the molecular mechanisms of heredity. These experiments are among the most elegant in science. And, just as in a good detective story, each discovery has led to new questions. But however erratic and lurching the course of the experimental journey may appear, our picture of heredity has become progressively clearer, the image more sharply defined.

14.1 The Nature of the Genetic Material

Learning Outcomes

1. *Describe the phenomenon of transformation.*
2. *Evaluate the evidence for DNA as genetic material.*

In the previous two chapters, you learned about the nature of inheritance and how genes, which contain the information to specify traits, are located on chromosomes. This finding led to the question of what part of the chromosome actually contains the genetic information. Specifically, biologists wondered about the chemical identity of the genetic information. They knew that chromosomes are composed primarily of both protein and DNA. Which of these organic molecules actually makes up the genes?

Starting in the late 1920s and continuing for about 30 years, a series of investigations addressed this question. DNA

consists of four chemically similar nucleotides. In contrast, protein contains 20 different amino acids that are much more chemically diverse than nucleotides. These characteristics seemed initially to indicate greater informational capacity in protein than in DNA.

However, experiments began to reveal evidence in favor of DNA. We describe three of those major findings in this section.

Griffith finds that bacterial cells can be transformed

The first clue came in 1928 with the work of the British microbiologist Frederick Griffith. Griffith was trying to make a vaccine that would protect against influenza, which was thought at the time to be caused by the bacteria *Streptococcus pneumoniae*. There are two forms of this bacteria: The normal virulent form that causes pneumonia, and a mutant, nonvirulent form that does not. The normal virulent form of this bacterium is referred to as the S form because it forms smooth colonies on a culture dish. The mutant, nonvirulent form, which lacks an enzyme needed to manufacture the polysaccharide coat, is called the R form because it forms rough colonies.

Griffith performed a series of simple experiments in which mice were infected with these bacteria, then monitored for disease symptoms (figure 14.1). Mice infected with the virulent S form died from pneumonia, whereas infection with the nonvirulent R form had no effect. This result shows that the polysaccharide coat is necessary for virulence. If the virulent S form is first heat-killed, infection does not harm the mice, showing that the coat itself is not sufficient to cause disease. Lastly, infecting mice with a mixture of heat-killed S form with live R form caused pneumonia and death in the mice. This was unexpected as neither treatment alone caused disease. Furthermore, high levels of live S form bacteria were found in the lungs of the dead mice.

Somehow, the information specifying the polysaccharide coat had passed from the dead, virulent S bacteria to the live, coatless R bacteria in the mixture, permanently altering the coatless R bacteria into the virulent S variety. Griffith called this transfer of virulence from one cell to another, **transformation.** Our modern interpretation is that genetic material was actually transferred between the cells.

Avery, MacLeod, and McCarty identify the transforming principle

The agent responsible for transforming *Streptococcus* went undiscovered until 1944. In a classic series of experiments, Oswald Avery and his coworkers Colin MacLeod and Maclyn McCarty identified the substance responsible for transformation in Griffith's experiment.

They first prepared the mixture of dead S *Streptococcus* and live R *Streptococcus* that Griffith had used. Then they removed as much of the protein as they could from their preparation, eventually achieving 99.98% purity. They found that despite the removal of nearly all protein, the transforming activity was not reduced.

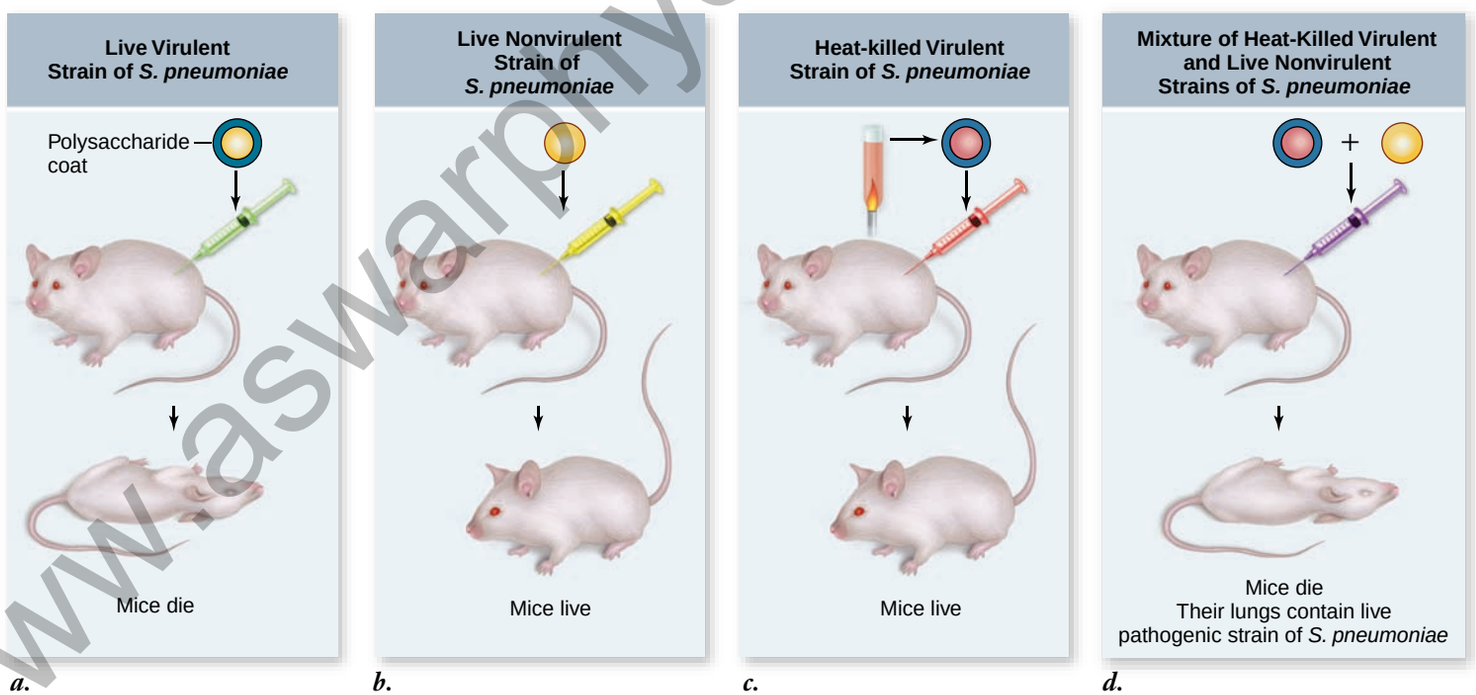


Figure 14.1 Griffith's experiment. Griffith was trying to make a vaccine against pneumonia and instead discovered transformation. ***a.*** Injecting live virulent bacteria into mice produces pneumonia. Injection of nonvirulent bacteria ***(b)*** or heat-killed virulent bacteria ***(c)*** had no effect. ***d.*** However, a mixture of heat-killed virulent and live nonvirulent bacteria produced pneumonia in the mice. This indicates the genetic information for virulence was transferred from dead, virulent cells to live, nonvirulent cells, transforming them from nonvirulent to virulent.

Moreover, the properties of this substance resembled those of DNA in several ways:

1. The elemental composition agreed closely with that of DNA.
2. When spun at high speeds in an ultracentrifuge, it migrated to the same level (density) as DNA.
3. Extracting lipids and proteins did not reduce transforming activity.
4. Protein-digesting enzymes did not affect transforming activity, nor did RNA-digesting enzymes.
5. DNA-digesting enzymes destroyed all transforming activity.

These experiments supported the identity of DNA as the substance transferred between cells by transformation and indicated that the genetic material, at least in this bacterial species, is DNA.

Hershey and Chase demonstrate that phage genetic material is DNA

Avery's results were not widely accepted at first because many biologists continued to believe that proteins were the repository of hereditary information. But additional evidence supporting Avery's conclusion was provided in 1952 by Alfred Hershey and Martha Chase, who experimented with viruses that infect bacteria. These viruses are called **bacteriophages,** or more simply, **phages.**

Viruses, described in more detail in chapter 27, are much simpler than cells; they generally consist of genetic material (DNA or RNA) surrounded by a protein coat. The phage used in these experiments is called a *lytic* phage because infection causes the cell to burst, or lyse. When such a phage infects a bacterial cell, it first binds to the cell's outer surface and then injects its genetic information into the cell. There, the viral genetic information is expressed by the bacterial cell's machinery, leading to production of thousands of new viruses. The buildup of viruses eventually causes the cell to lyse, releasing progeny phage.

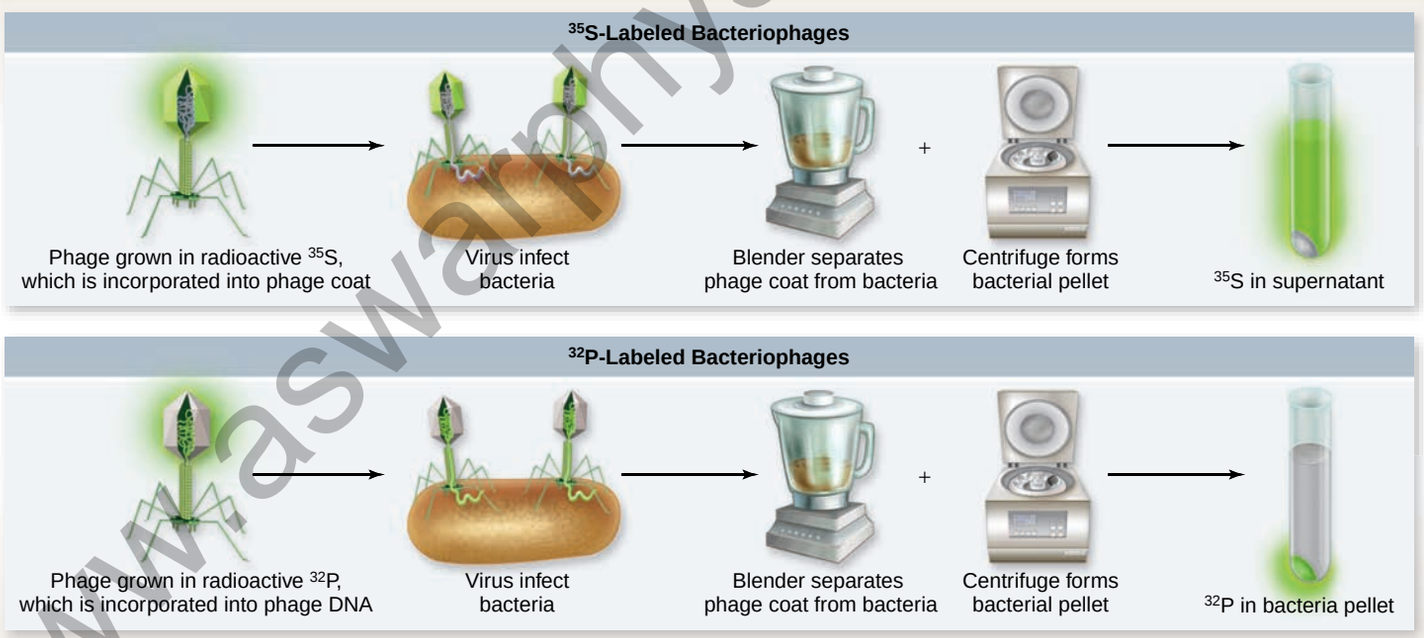
The phage used by Hershey and Chase contains only DNA and protein, and therefore it provides the simplest possible system to differentiate the roles of DNA and protein. Hershey and Chase set out to identify the molecule that the phage injects into the bacterial cells. To do this, they needed a method to label both DNA and protein in unique ways that would allow them to be distinguished. Nucleotides

SCIENTIFIC THINKING

Hypothesis: *DNA is the genetic material in bacteriophage.*

Prediction: *The phage life cycle requires reprogramming the cell to make phage proteins. The information for this must be introduced into the cell during infection.*

Test: *DNA can be specifically labeled using radioactive phosphate (^{32}P), and protein can be specifically labeled using radioactive sulfur (^{35}S) . Phage are grown on either ^{35}S or ^{32}P, then used to infect cells in two experiments. The phage heads remain attached to the outside of the cell and can be removed by brief agitation in a blender. The cell suspension can be collected by centrifugation, leaving the phage heads in the supernatant.*



Result: *When the experiment is done, only ^{32}P makes it into the cell in any significant quantity.*

Conclusion: *Thus, DNA must be the molecule that is used to reprogram the cell.*

Further Experiments: *How does this experiment complement or extend the work of Avery on the identity of the transforming principle?*

Figure 14.2 **Hershey–Chase experiment showed DNA is genetic material for phage.**

contain phosphorus, but proteins do not, and some amino acids contain sulfur, but DNA does not. Thus, the radioactive ^{32}P isotope can be used to label DNA specifically, and the isotope ^{35}S can be used to label proteins specifically. The two isotopes are easily distinguished based on the particles they emit when they decay.

Two experiments were performed (figure 14.2). In one, viruses were grown on a medium containing ^{32}P, which was incorporated into DNA; in the other, viruses were grown on medium containing ^{35}S, which was incorporated into coat proteins. Each group of labeled viruses was then allowed to infect separate bacterial cultures.

After infection, the bacterial cell suspension was agitated in a blender to remove the infecting viral particles from the surfaces of the bacteria. This step ensured that only the part of the virus that had been injected into the bacterial cells—that is, the genetic material—would be detected.

Each bacterial suspension was then centrifuged to produce a pellet of cells for analysis. In the ^{32}P experiment, a large amount of radioactive phosphorus was found in the cell pellet, but in the ^{35}S experiment, very little radioactive sulfur was found in the pellet (see figure 14.2). Hershey and Chase deduced that DNA, and not protein, constituted the genetic information that viruses inject into bacteria.

Learning Outcomes Review 14.1

Experiments with pneumonia-causing bacteria showed that virulence could be passed from one cell to another, a phenomenon termed transformation. When the factor responsible for transformation was purified, it was shown to be DNA. Labeling experiments with phage also indicated that the genetic material was DNA and not protein.

- ***Why was protein an attractive candidate for the genetic material?***

14.2 *DNA Structure*

Learning Outcomes

1. ***Describe the data available to Watson and Crick.***
2. ***Explain the details of the Watson and Crick structure.***
3. ***Explain the importance of complementarity for DNA structure and function.***

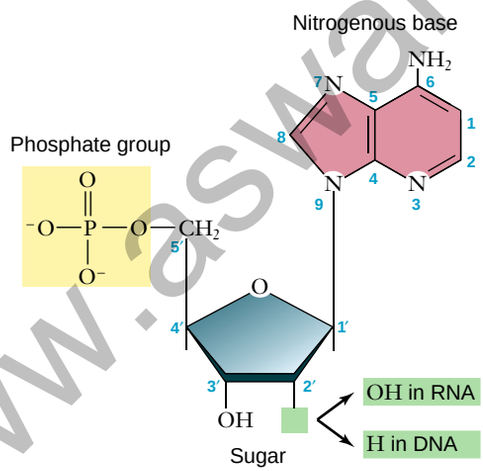
A Swiss chemist, Friedrich Miescher, discovered DNA in 1869, only four years after Mendel's work was published—although it is unlikely that Miescher knew of Mendel's experiments.

Miescher extracted a white substance from the nuclei of human cells and fish sperm. The proportion of nitrogen and phosphorus in the substance was different from that found in any other known constituent of cells, which convinced Miescher that he had discovered a new biological substance. He called this substance "nuclein" because it seemed to be specifically associated with the nucleus. Because Miescher's nuclein was slightly acidic, it came to be called *nucleic acid*.

DNA's components were known, but its three-dimensional structure was a mystery

Although the three-dimensional structure of the DNA molecule was not elucidated until Watson and Crick, it was known that it contained three main components (figure 14.3):

1. a five-carbon sugar
2. a phosphate (PO_4) group



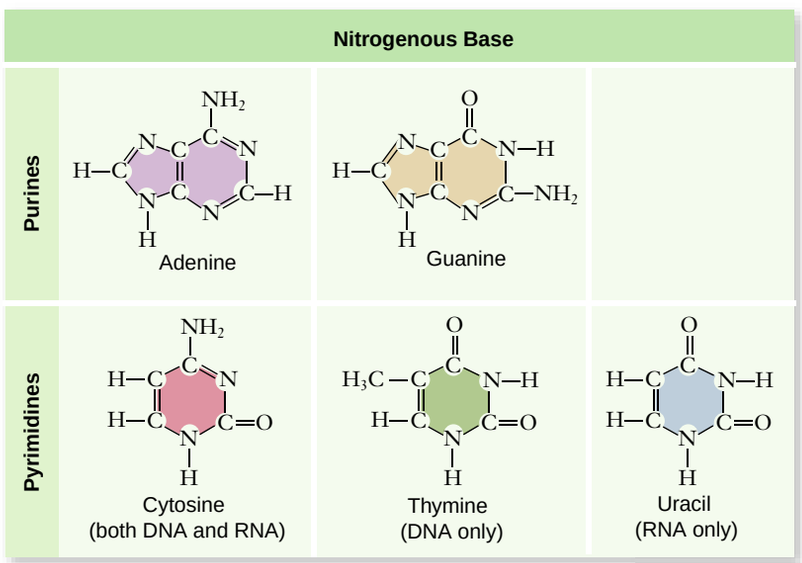


Figure 14.3 Nucleotide subunits of DNA and RNA. The nucleotide subunits of DNA and RNA are composed of three components: a five-carbon sugar (deoxyribose in DNA and ribose in RNA); a phosphate group; and a nitrogenous base (either a purine or a pyrimidine).

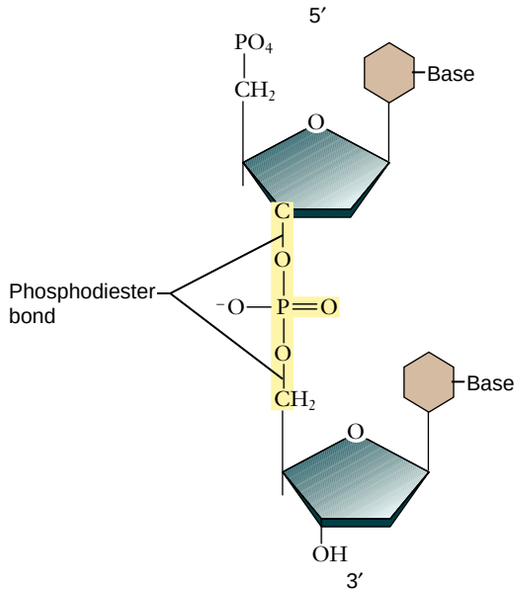


Figure 14.4 **A phosphodiester bond.**

3. a nitrogen-containing (nitrogenous) base. The base may be a **purine** (adenine, A, or guanine, G), a two-ringed structure; or a **pyrimidine** (thymine, T, or cytosine, C), a single-ringed structure. RNA contains the pyrimidine uracil (U) in place of thymine.

The convention in organic chemistry is to number the carbon atoms of a molecule and then to use these numbers to refer to any functional group attached to a carbon atom (see chapter 3). In the ribose sugars found in nucleic acids, four of the carbon atoms together with an oxygen atom form a five-membered ring. As illustrated in figure 14.3, the carbon atoms are numbered 1′ to 5′, proceeding clockwise from the oxygen atom; the prime symbol (′) indicates that the number refers to a carbon in a sugar rather than to the atoms in the bases attached to the sugars.

Under this numbering scheme, the phosphate group is attached to the 5′ carbon atom of the sugar, and the base is attached to the 1′ carbon atom. In addition, a free hydroxyl (—OH) group is attached to the 3′ carbon atom.

The 5′ phosphate and 3′ hydroxyl groups allow DNA and RNA to form long chains of nucleotides by the process of dehydration synthesis (see chapter 3). The linkage is called a **phosphodiester bond** because the phosphate group is now linked to the two sugars by means of a pair of ester bonds (figure 14.4). Many thousands of nucleotides can join together via these linkages to form long nucleic acid polymers.

Linear strands of DNA or RNA, no matter how long, almost always have a free 5′ phosphate group at one end and a free 3′ hydroxyl group at the other. Therefore, every DNA and RNA molecule has an intrinsic polarity, and we can refer unambiguously to each end of the molecule. By convention, the sequence of bases is usually written in the 5′-to-3′ direction.

Chargaff, Franklin, and Wilkins obtained some structural evidence

To understand the model that Watson and Crick proposed, we need to review the evidence that they had available to construct their model.

Chargaff's rules

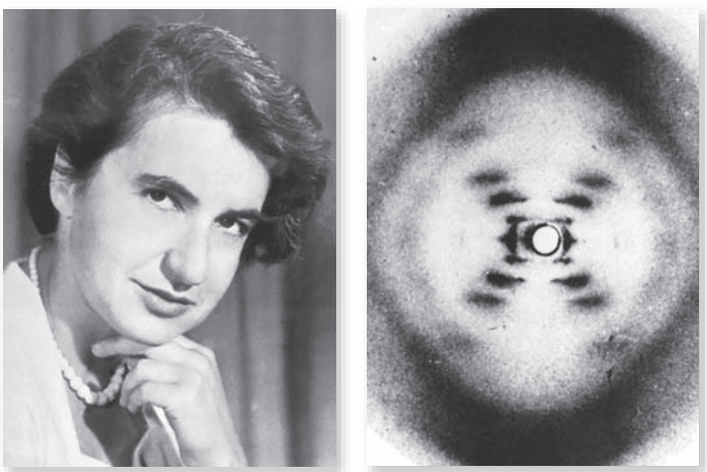
A careful study carried out by Erwin Chargaff showed that the nucleotide composition of DNA molecules varied in complex ways, depending on the source of the DNA. This strongly suggested that DNA was not a simple repeating polymer and that it might have the information-encoding properties genetic material requires. Despite DNA's complexity, however, Chargaff observed an important underlying regularity in the ratios of the bases found in native DNA: *The amount of adenine present in DNA always equals the amount of thymine, and the amount of guanine always equals the amount of cytosine.* These findings are commonly referred to as *Chargaff's rules:*

1. The proportion of A always equals that of T, and the proportion of G always equals that of C, or: A = T, and G = C.
2. It follows that there is always an equal proportion of purines (A and G) and pyrimidines (C and T).

As mounting evidence indicated that DNA stored the hereditary information, investigators began to puzzle over how such a seemingly simple molecule could carry out such a complex coding function.

Franklin: X-ray diffraction patterns of DNA

Another line of evidence provided more direct information about the possible structure of DNA. The British chemist Rosalind Franklin (figure 14.5*a*) used the technique of X-ray diffraction to analyze DNA. In X-ray diffraction, a molecule is bombarded with a beam of X-rays. The rays are bent, or



a. ***b.***

Figure 14.5 **Rosalind Franklin's X-ray diffraction patterns.** ***a.*** Rosalind Franklin. ***b.*** This X-ray diffraction photograph of DNA fibers, made in 1953 by Rosalind Franklin was interpreted to show the helical structure of DNA.

Figure 14.6 The DNA double helix. James Watson *(left)* and Francis Crick *(right)* deduced the structure of DNA in 1953 from Chargaff's rules, knowing the proper tautomeric forms of the bases and using Franklin's diffraction studies.

diffracted, by the molecules they encounter, and the diffraction pattern is recorded on photographic film. The patterns resemble the ripples created by tossing a rock into a smooth lake (figure 14.5*b*). When analyzed mathematically, the diffraction pattern can yield information about the three-dimensional structure of a molecule.

X-ray diffraction works best on substances that can be prepared as perfectly regular crystalline arrays. At the time Franklin conducted her analysis, it was impossible to obtain true crystals of natural DNA, so she had to use DNA in the form of fibers. Maurice Wilkins, another researcher working in the same laboratory, had been able to prepare more uniformly oriented DNA fibers than anyone else at the time. Using these fibers, Franklin succeeded in obtaining crude diffraction information on natural DNA. The diffraction patterns she obtained suggested that the DNA molecule had the shape of a helix, or corkscrew, with a consistent diameter of about 2 nm and a complete helical turn every 3.4 nm.

Tautomeric forms of bases

One piece of evidence important to Watson and Crick was the form of the bases themselves. Because of the alternating double and single bonds in the bases, they actually exist in equilibrium between two different forms when in solution. The different forms have to do with keto (C═O) versus enol (C—OH) groups and amino (—NH_2) versus imino (═NH) groups that are attached to the bases. These structural forms are called *tautomers*.

The importance of this distinction is that the two forms exhibit very different hydrogen-bonding possibilities. The predominant forms of the bases contain the keto and amino groups (see figure 14.3), but a prominent biochemistry text of the time actually contained the opposite, and incorrect, information. Legend has it that Watson learned the correct forms while having lunch with a biochemist friend.

The Watson–Crick model fits the available evidence

Learning informally of Franklin's results before they were published in 1953, James Watson and Francis Crick, two young investigators at Cambridge University, quickly worked out a likely structure for the DNA molecule (figure 14.6), which we now know was substantially correct. Watson and Crick did not perform a single experiment themselves related to DNA structure; rather, they built detailed molecular models based on the information available.

The key to the model was their understanding that each DNA molecule is actually made up of *two* chains of nucleotides that are intertwined—the double helix.

The phosphodiester backbone

The two strands of the double helix are made up of long polymers of nucleotides, and as described earlier, each strand is made up of repeating sugar and phosphate units joined by phosphodiester bonds (figure 14.7). We call this the *phosphodiester backbone* of the

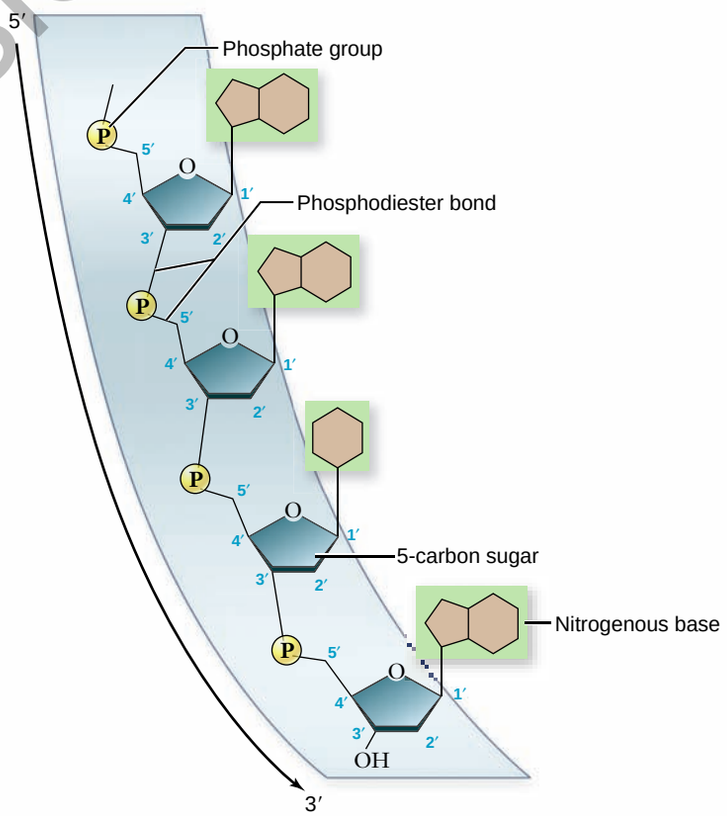


Figure 14.7 Structure of a single strand of DNA. The phosphodiester backbone is composed of alternating sugar and phosphate groups. The bases are attached to each sugar.

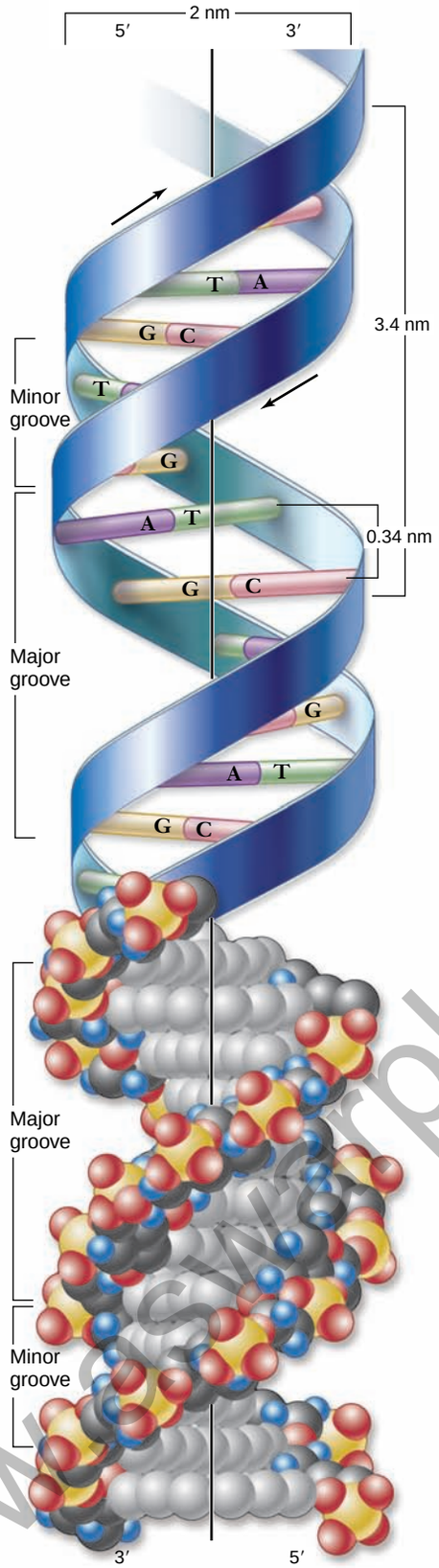


Figure 14.8 The double helix. Shown with the phosphodiester backbone as a ribbon on top and a space-filling model on the bottom. The bases protrude into the interior of the helix where they hold it together by base-pairing. The backbone forms two grooves, the larger major groove and the smaller minor groove.

molecule. The two strands of the backbone are then wrapped about a common axis forming a double helix (figure 14.8). The helix is often compared to a spiral staircase, in which the two strands of the double helix are the handrails on the staircase.

Complementarity of bases

Watson and Crick proposed that the two strands were held together by formation of hydrogen bonds between bases on opposite strands. These bonds would result in specific **base-pairs:** Adenine (A) can form two hydrogen bonds with thymine (T) to form an A–T base-pair, and guanine (G) can form three hydrogen bonds with cytosine (C) to form a G–C base-pair (figure 14.9).

Note that this configuration also pairs a two-ringed purine with a single-ringed pyrimidine in each case, so that the diameter of each base-pair is the same. This consistent diameter is indicated by the X-ray diffraction data.

We refer to this pattern of base-pairing as *complementary*, which means that although the strands are not identical, they each can be used to specify the other by base-pairing. If the sequence of one strand is ATGC, then the complementary strand sequence must be TACG. This characteristic becomes critical for DNA replication and expression, as you will see later in this chapter.

The Watson–Crick model also explained Chargaff's results: In a double helix, adenine forms two hydrogen bonds with thymine, but it will not form hydrogen bonds properly with cytosine. Similarly, guanine forms three hydrogen bonds with cytosine, but it will not form hydrogen bonds properly with thymine. Because of this base-pairing, adenine and thymine always occur in the same proportions in any DNA molecule, as do guanine and cytosine.

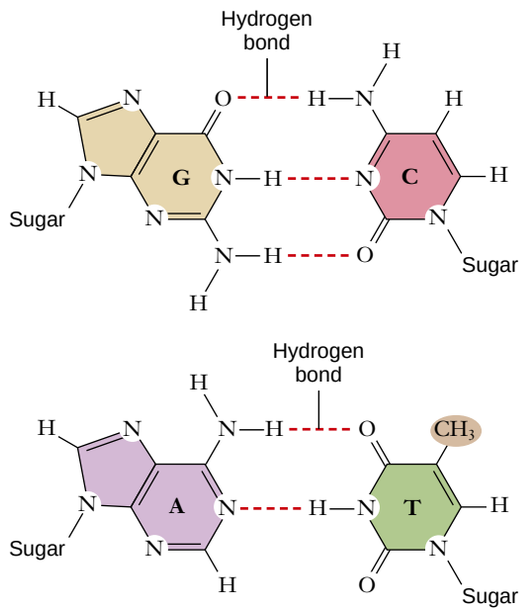


Figure 14.9 Base-pairing holds strands together. The hydrogen bonds that form between A and T and between G and C are shown with dashed lines. These produce AT and GC base-pairs that hold the two strands together. This always pairs a purine with a pyrimidine, keeping the diameter of the double helix constant.

Inquiry question

Does the Watson–Crick model account for all of the data discussed in the text?

Antiparallel configuration

As stated earlier, a single phosphodiester strand has an inherent polarity, meaning that one end terminates in a 3′ OH and the other end terminates in a 5′ PO_4. Strands are thus referred to as having either a 5′-to-3′ or a 3′-to-5′ polarity. Two strands could be put together in two ways: with the polarity the same in each (parallel) or with the polarity opposite (antiparallel). Native double-stranded DNA always has the antiparallel configuration, with one strand running 5′ to 3′ and the other running 3′ to 5′ (see figure 14.8). In addition to its complementarity, this antiparallel nature also has important implications for DNA replication.

The Watson–Crick DNA molecule

In the Watson and Crick model, each DNA molecule is composed of two complementary phosphodiester strands that each form a helix with a common axis. These strands are antiparallel, with the bases extending into the interior of the helix. The bases from opposite strands form base-pairs with each other to join the two complementary strands (see figures 14.8 and 14.9).

Although the hydrogen bonds between each individual base-pair are low-energy bonds, the sum of bonds between the many base-pairs of the polymer has enough energy that the entire molecule is stable. To return to our spiral staircase analogy—the backbone is the handrails, the base-pairs are the steps.

Although the Watson–Crick model provided a rational structural for DNA, researchers had to answer further questions about how DNA could be replicated, a crucial step in cell division, and also about how cells could repair damaged or otherwise altered DNA. We explore these questions in the rest of this chapter. (In the following chapter, we continue with the genetic code and the connection between the code and protein synthesis.)

Learning Outcomes Review 14.2

Chargaff showed that in DNA, the amount of adenine was equal to the amount of thymine, and the amount of guanosine was equal to that of cytosine. X-ray diffraction studies by Franklin and Wilkins indicated that DNA formed a helix. Watson and Crick built a model consisting of two antiparallel strands wrapped in a helix about a common axis. The two strands are held together by hydrogen bonds between the bases: adenine pairs with thymine and guanine pairs with cytosine. The two strands are thus complementary to each other.

- ***Why was information about the proper tautomeric form of the bases critical?***

14.3 *Basic Characteristics of DNA Replication*

Learning Outcomes

1. ***Explain the basic mechanism of DNA replication.***
2. ***Describe the requirements for DNA replication.***

The accurate replication of DNA prior to cell division is a basic and crucial function. Research has revealed that this complex process requires the participation of a large number of cellular proteins. Before geneticists could look for these details, however, they needed to perform some groundwork on the general mechanisms.

Meselson and Stahl demonstrate the semiconservative mechanism

The Watson–Crick model immediately suggested that the basis for copying the genetic information is complementarity. One chain of the DNA molecule may have any conceivable base sequence, but this sequence completely determines the sequence of its partner in the duplex.

In replication, the sequence of parental strands must be duplicated in daughter strands. That is, one parental helix with two strands must yield two daughter helices with four strands. The two daughter molecules are then separated during the course of cell division.

Three models of DNA replication are possible (figure 14.10):

1. In a *conservative model*, both strands of the parental duplex would remain intact (conserved), and new DNA copies would consist of all-new molecules. Both daughter strands would contain all-new molecules.

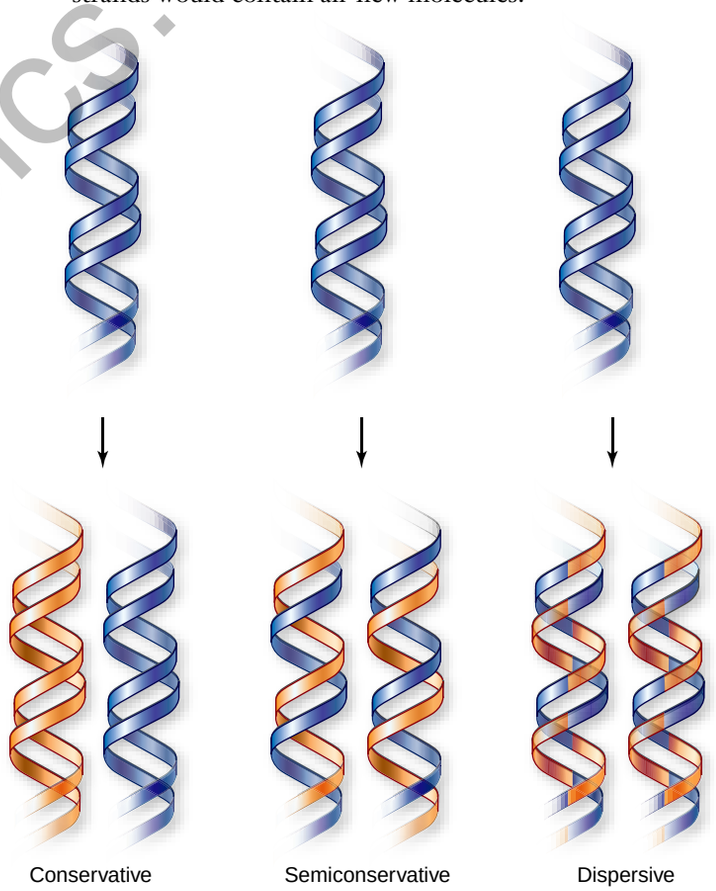


Figure 14.10 Three possible models for DNA replication. The conservative model produces one entirely new molecule and conserves the old. The semiconservative model produces two hybrid molecules of old and new strands. The dispersive model produces hybrid molecules with each strand a mixture of old and new.

2. In a **semiconservative model,** one strand of the parental duplex remains intact in daughter strands (semiconserved); a new complementary strand is built for each parental strand consisting of new molecules. Daughter strands would consist of one parental strand and one newly synthesized strand.
3. In a *dispersive model,* copies of DNA would consist of mixtures of parental and newly synthesized strands; that is, the new DNA would be dispersed throughout each strand of both daughter molecules after replication.

Notice that these three models suggest general mechanisms of replication, without specifying any molecular details of the process.

The Meselson–Stahl experiment

The three models for DNA replication were evaluated in 1958 by Matthew Meselson and Franklin Stahl. To distinguish between these models, they labeled DNA and then followed the labeled DNA through two rounds of replication (figure 14.11).

The label Meselson and Stahl used was a heavy isotope of nitrogen (^{15}N), not a radioactive label. Molecules containing ^{15}N have a greater density than those containing the common ^{14}N isotope. Ultracentrifugation can be used to separate molecules that have different densities.

Bacteria were grown in a medium containing ^{15}N, which became incorporated into the bases of the bacterial DNA. After several generations, the DNA of these bacteria was denser than that of bacteria grown in a medium containing the normally available ^{14}N. Meselson and Stahl then transferred the bacteria from the ^{15}N medium to ^{14}N medium and collected the DNA at various time intervals.

The DNA for each interval was dissolved in a solution containing a heavy salt, cesium chloride. This solution was spun at very high speeds in an ultracentrifuge. The enormous centrifugal forces caused cesium ions to migrate toward the bottom of the centrifuge tube, creating a gradient of cesium concentration, and thus of density. Each DNA strand floated or sank in the gradient until it reached the point at which its density exactly matched the density of the cesium at that location. Because ^{15}N strands are denser than ^{14}N strands, they migrated farther down the tube.

The DNA collected immediately after the transfer of bacteria to new ^{14}N medium was all of one density equal to that of ^{15}N DNA alone. However, after the bacteria completed a first round of DNA replication, the density of their DNA had decreased to a value intermediate between ^{14}N DNA alone and ^{15}N DNA. After the second round of replication, two density classes of DNA were observed: one intermediate and one equal to that of ^{14}N DNA (see figure 14.11).

Interpretation of the Meselson–Stahl findings

Meselson and Stahl compared their experimental data with the results that would be predicted on the basis of the three models.

1. The conservative model was not consistent with the data because after one round of replication, two densities should have been observed: DNA strands would either be all-heavy (parental) or all-light (daughter). This model is rejected.
2. The semiconservative model is consistent with all observations: After one round of replication, a single

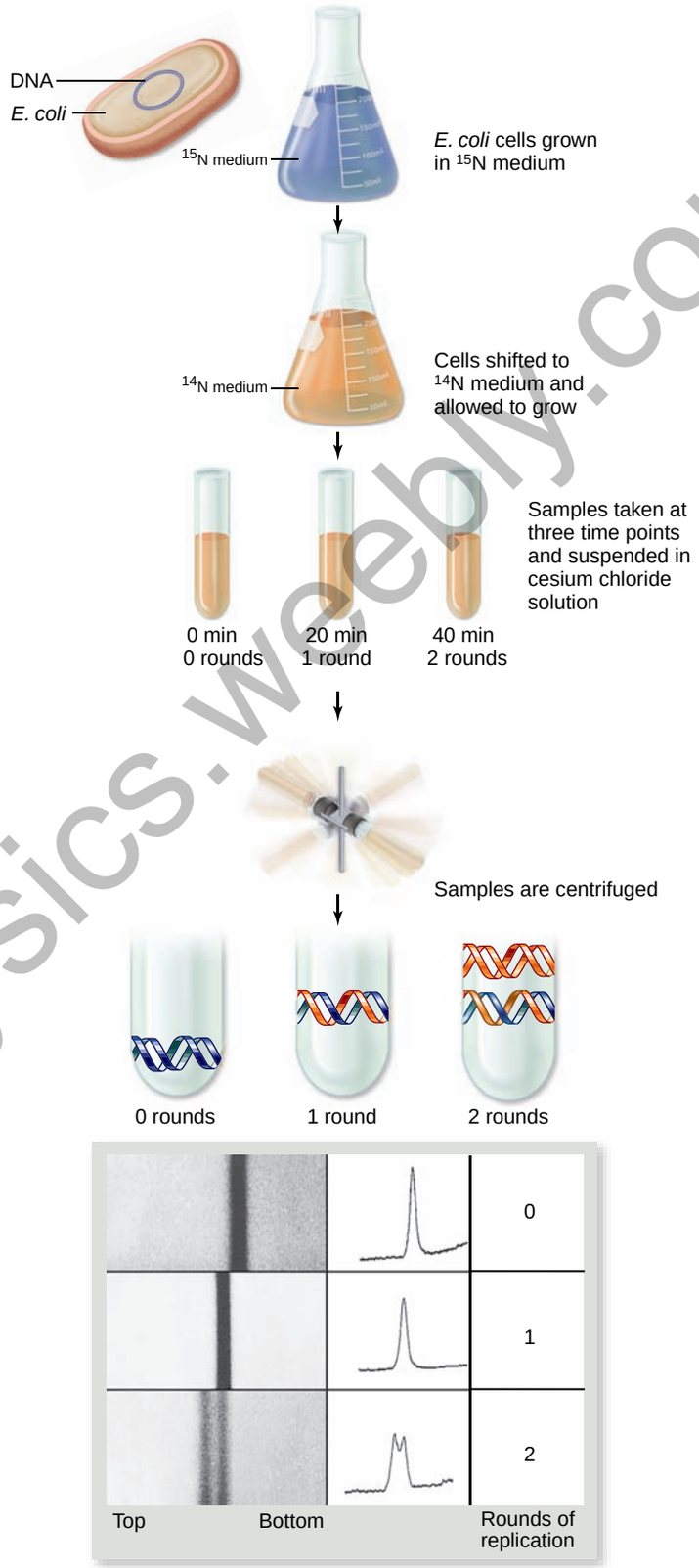


Figure 14.11 The Meselson–Stahl experiment. Bacteria grown in heavy ^{15}N medium are shifted to light ^{14}N medium and grown for two rounds of replication. Samples are taken at time points corresponding to zero, one, and two rounds of replication and centrifuged in cesium chloride to form a gradient. The actual data are shown at the bottom with the interpretation of semiconservative replication shown schematically.

density would be predicted because all DNA molecules would have a light strand and a heavy strand. After two rounds of replication, half of the molecules would have two light strands, and half would have a light strand and a heavy strand—and so two densities would be observed. Therefore, the results support the semiconservative model.

3. The dispersive model was consistent with the data from the first round of replication, because in this model, every DNA helix would consist of strands that are mixtures of ½ light (new) and ½ heavy (old) molecules. But after two rounds of replication, the dispersive model would still yield only a single density; DNA strands would be composed of ¾ light and ¼ heavy molecules. Instead, two densities were observed. Therefore, this model is also rejected.

The basic mechanism of DNA replication is semiconservative. At the simplest level, then, DNA is replicated by opening up a DNA helix and making copies of both strands to produce two daughter helices, each consisting of one old strand and one new strand.

DNA replication requires a template, nucleotides, and a polymerase enzyme

Replication requires three things: something to copy, something to do the copying, and the building blocks to make the copy. The parental DNA molecules serve as a template, enzymes perform the actions of copying the template, and the building blocks are nucleoside triphosphates.

The process of replication can be thought of as having a beginning where the process starts; a middle where the majority of building blocks are added; and an end where the process is finished. We use the terms *initiation*, *elongation*, and *termination* to describe a biochemical process. Although this may seem overly simplistic, in fact, discrete functions are usually required for initiation and termination that are not necessary for elongation.

A number of enzymes work together to accomplish the task of assembling a new strand, but the enzyme that actually matches the existing DNA bases with complementary nucleotides and then links the nucleotides together to make the new strand is **DNA polymerase** (figure 14.12). All DNA

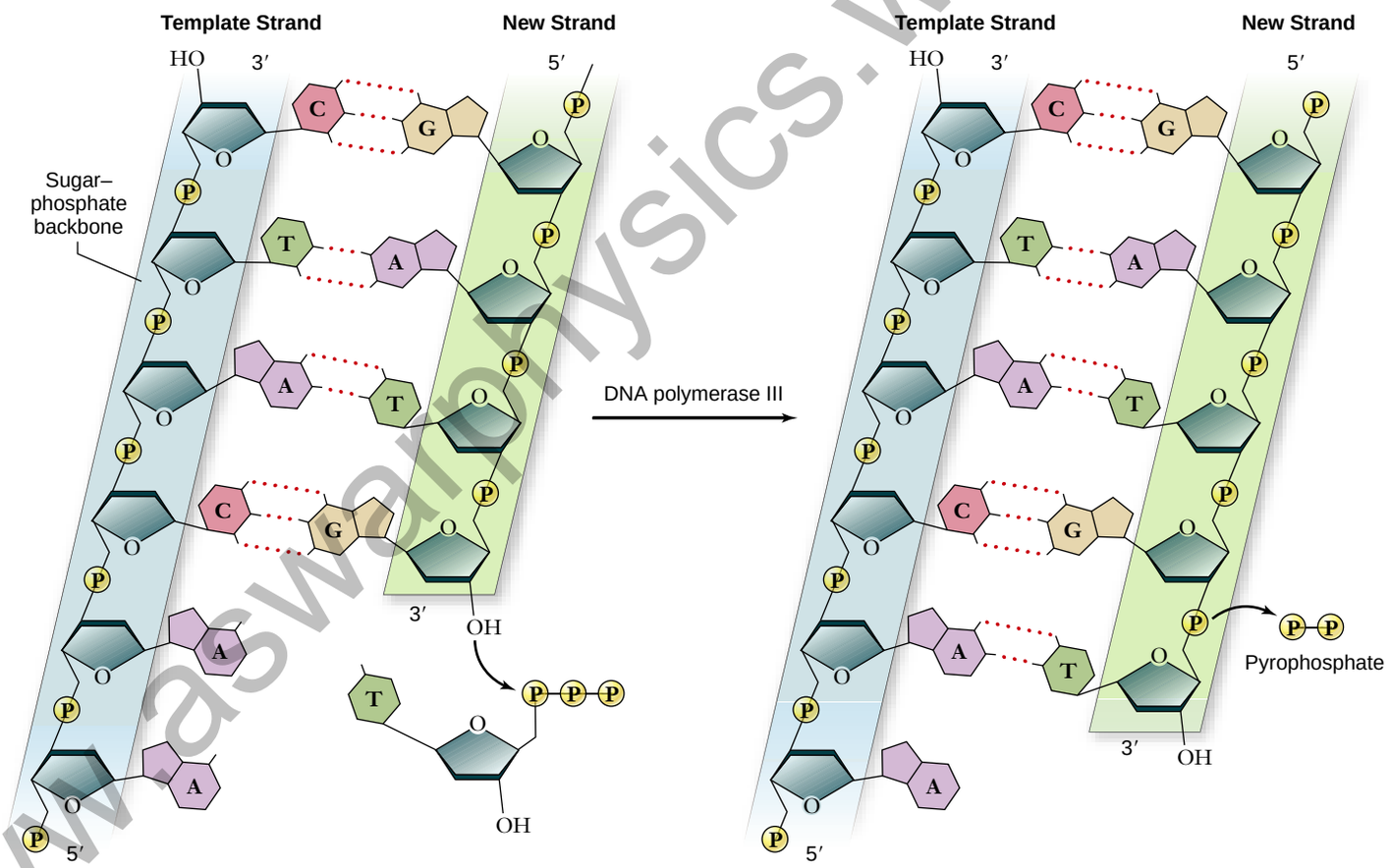
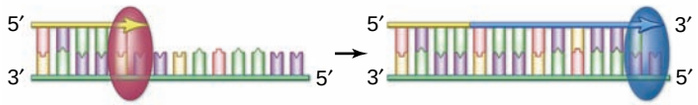


Figure 14.12 Action of DNA polymerase. DNA polymerases add nucleotides to the 3′ end of a growing chain. The nucleotide added depends on the base that is in the template strand. Each new base must be complementary to the base in the template strand. With the addition of each new nucleoside triphosphate, two of its phosphates are cleaved off as pyrophosphate.

Inquiry question

Why do you think it is important that the sugar–phosphate backbone of DNA is held together by covalent bonds, and the cross-bridges between the two strands are held together by hydrogen bonds?

polymerases that have been examined have several common features. They all add new bases to the 3′ end of existing strands. That is, they synthesize in a 5′-to-3′ direction by extending a strand base-paired to the template. All DNA polymerases also require a *primer* to begin synthesis; they cannot begin without a strand of RNA or DNA base-paired to the template. RNA polymerases do not have this requirement, so they usually synthesize the primers.



RNA polymerase makes primer DNA polymerase extends primer

Learning Outcomes Review 14.3

Meselson and Stahl showed that the basic mechanism of replication is semiconservative: Each new DNA helix is composed of one old strand and one new strand. The process of replication requires a template to copy, nucleoside triphosphate building blocks, and the enzyme DNA polymerase. DNA polymerases synthesize DNA in a 5′-to-3′ direction from a primer, usually RNA.

- ***In the Meselson–Stahl experiment, what would the results be if the DNA was denatured prior to separation by ultracentrifugation?***

14.4 Prokaryotic Replication

Learning Outcomes

1. *Describe the functions of E. coli DNA polymerases.*
2. *Explain why replication is discontinuous on one strand.*
3. *Diagram the functions found at the replication fork.*

To build up a more detailed picture of replication, we first concentrate on prokaryotic replication using *E. coli* as a model. We can then look at eukaryotic replication primarily in how it differs from the prokaryotic system.

Prokaryotic replication starts at a single origin

Replication in *E. coli* initiates at a specific site, the origin (called *oriC*), and ends at a specific site, the terminus. The sequence of *oriC* consists of repeated nucleotides that bind an initiator protein and an AT-rich sequence that can be opened easily during initiation of replication. (A–T base-pairs have only two hydrogen bonds, compared with the three hydrogen bonds in G–C base-pairs.)

After initiation, replication proceeds bidirectionally from this unique origin to the unique terminus (figure 14.13). We call the DNA controlled by an origin a **replicon.** In this case, the chromosome plus the origin forms a single replicon.

E. coli has at least three different DNA polymerases

As mentioned earlier, DNA polymerase refers to a group of enzymes responsible for the building of a new DNA strand from the template. The first DNA polymerase isolated in *E. coli* was given the name **DNA polymerase I (Pol I).** At first, investigators assumed this polymerase was responsible for the bulk synthesis of DNA during replication. A mutant was isolated, however, that had no Pol I activity, but could still replicate its chromosome. Two additional polymerases were isolated from this strain of *E. coli* and were named **DNA polymerase II (Pol II)** and **DNA polymerase III (Pol III).** As with all other known polymerases, all three of these enzymes synthesize polynucleotide strands only in the 5′-to-3′ direction and require a primer.

Many DNA polymerases have additional enzymatic activity that aids their function. This activity is a nuclease activity, or the ability to break phosphodiester bonds between nucleotides. Nucleases are classified as either **endonucleases** (which cut DNA internally) or **exonucleases** (which chew away at an end of DNA). DNA Pol I, Pol II, and Pol III have 3′-to-5′ exonuclease activity, which serves as a proofreading function because it allows the enzyme to remove a mispaired base. In addition, the DNA Pol I enzyme also has a 5′-to-3′ exonuclease activity, the importance of which will become clear shortly.

The three different polymerases have different roles in the replication process. DNA Pol III is the main replication enzyme; it is responsible for the bulk of DNA synthesis. DNA Pol I acts on the lagging strand to remove primers and replace them with DNA. The Pol II enzyme does not appear to play a role in replication but is involved in DNA repair processes.

For many years, these three polymerases were thought to be the only DNA polymerases in *E. coli*, but recently several

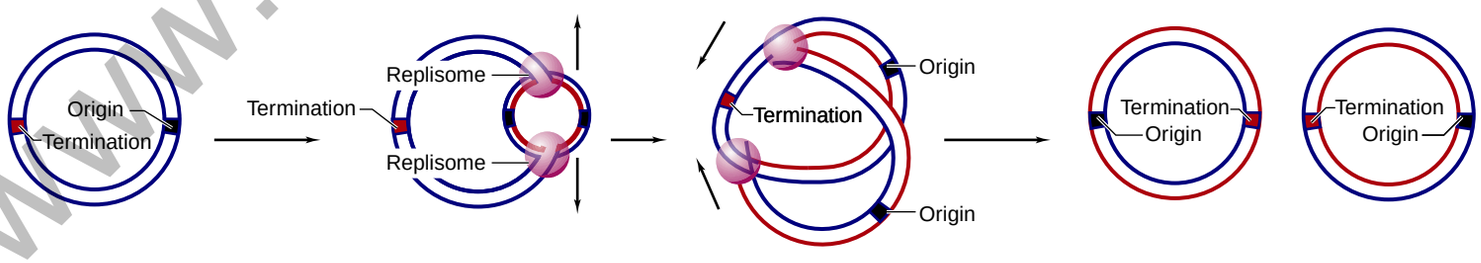


Figure 14.13 Replication is bidirectional from a unique origin. Replication initiates from a unique origin. Two separate replisomes are loaded onto the origin and initiate synthesis in the opposite directions on the chromosome. These two replisomes continue in opposite directions until they come to a unique termination site.

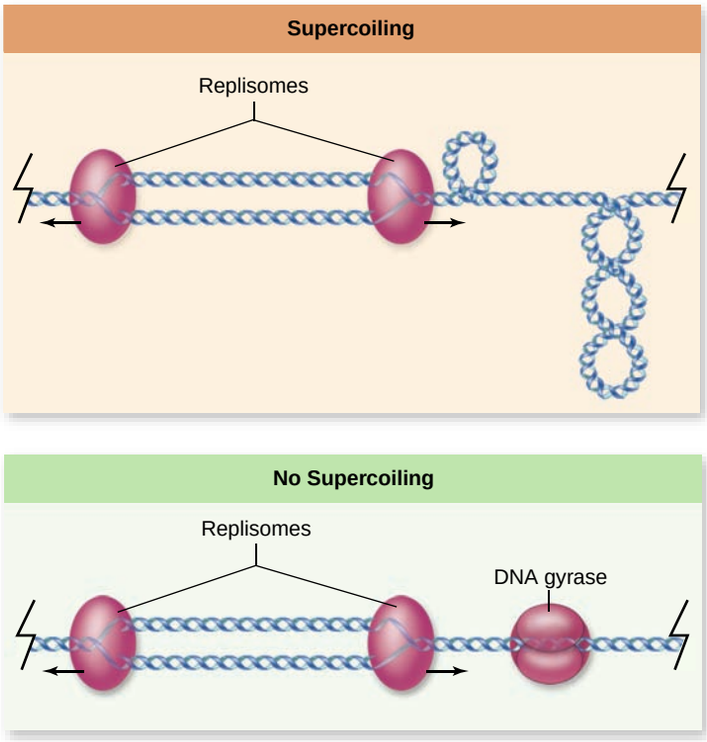


Figure 14.14 Unwinding the helix causes torsional strain. If the ends of a linear DNA molecule are constrained, as they are in the cell, unwinding the helix produces torsional strain. This can cause the double helix to further coil in space (supercoiling). The enzyme DNA gyrase can relieve supercoiling.

new ones have been identified. There are now five known polymerases, although not all are active in DNA replication.

Unwinding DNA requires energy and causes torsional strain

Although some DNA polymerases can unwind DNA as they synthesize new DNA, another class of enzymes has the single function of unwinding DNA strands to make this process more efficient. Enzymes that use energy from ATP to unwind the DNA template are called **helicases.**

The single strands of DNA produced by helicase action are unstable because the process exposes the hydrophobic bases to water. Cells solve this problem by using a protein, called single-strand-binding protein (SSB), to coat exposed single strands.

The unwinding of the two strands introduces torsional strain in the DNA molecule. Imagine two rubber bands twisted together. If you now unwind the rubber bands, what happens? The rubber bands, already twisted about each other, will further coil in space. When this happens with a DNA molecule it is called **supercoiling** (figure 14.14). The branch of mathematics that studies how forms twist and coil in space is called *topology*, and therefore we describe this coiling of the double helix as the *topological state* of DNA. This state describes how the double helix itself coils in space. You have already seen an example of this coiling with DNA wrapped about histone proteins in the nucleosomes of eukaryotic chromosomes (see chapter 10).

Enzymes that can alter the topological state of DNA are called **topoisomerases.** Topoisomerase enzymes act to relieve the torsional strain caused by unwinding and to prevent this supercoiling from happening. **DNA gyrase** is the topoisomerase involved in DNA replication (see figure 14.14).

Replication is semidiscontinuous

Earlier, DNA was described as being antiparallel—meaning that one strand runs in the 3′-to-5′ direction, and its complementary strand runs in the 5′-to-3′ direction. The antiparallel nature of DNA combined with the nature of the polymerase enzymes puts constraints on the replication process. Because polymerases can synthesize DNA in only one direction, and the two DNA strands run in opposite directions, polymerases on the two strands must be synthesizing DNA in opposite directions (figure 14.15).

The requirement of DNA polymerases for a primer means that on one strand primers need to be added as the helix

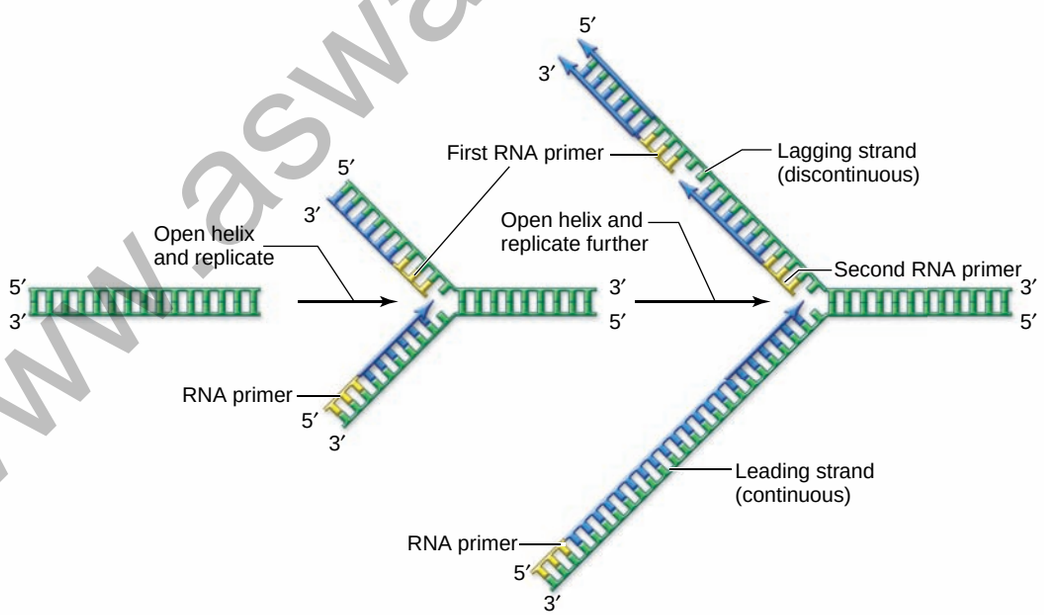


Figure 14.15 Replication is semidiscontinuous. The 5′-to-3′ synthesis of the polymerase and the antiparallel nature of DNA mean that only one strand, the leading strand, can be synthesized continuously. The other strand, the lagging strand, must be made in pieces, each with its own primer.

is opened up (see figure 14.15). This means that one strand can be synthesized in a continuous fashion from an initial primer, but the other strand must be synthesized in a discontinuous fashion with multiple priming events and short sections of DNA being assembled. The strand that is continuous is called the **leading strand,** and the strand that is discontinuous is the **lagging strand.** DNA fragments synthesized on the lagging strand are named **Okazaki fragments** in honor of the man who first experimentally demonstrated discontinuous synthesis. They introduce a need for even more enzymatic activity on the lagging strand, as is described next.

Synthesis occurs at the replication fork

The partial opening of a DNA helix to form two single strands has a forked appearance, and is thus called the **replication fork.** All of the enzymatic activities that we have discussed plus a few more are found at the replication fork (table 14.1). Synthesis on the leading strand and on the lagging strand proceed in different ways, however.

Priming

The primers required by DNA polymerases during replication are synthesized by the enzyme *DNA primase*. This enzyme is an RNA polymerase that synthesizes short stretches of RNA 10–20 bp (base-pairs) long that function as primers for DNA polymerase. Later on, the RNA primer is removed and replaced with DNA.

Leading-strand synthesis

Synthesis on the leading strand is relatively simple. A single priming event is required, and then the strand can be extended indefinitely by the action of DNA Pol III. If the enzyme remains attached to the template, it can synthesize around the entire circular *E. coli* chromosome.

The ability of a polymerase to remain attached to the template is called *processivity*. The Pol III enzyme is a large multisubunit enzyme that has high processivity due to the action of one subunit of the enzyme, called the β *subunit* (figure 14.16*a*).

The β subunit is made up of two identical protein chains that come together to form a circle. This circle can be loaded onto the template like a clamp to hold the Pol II enzyme to the DNA (figure 14.16*b*). This structure is therefore referred to as the "sliding clamp," and a similar structure is found in eukaryotic polymerases as well. For the clamp to function, it must be opened and then closed around the DNA. A multisubunit protein called the clamp loader accomplishes this task. This function is also found in eukaryotes.

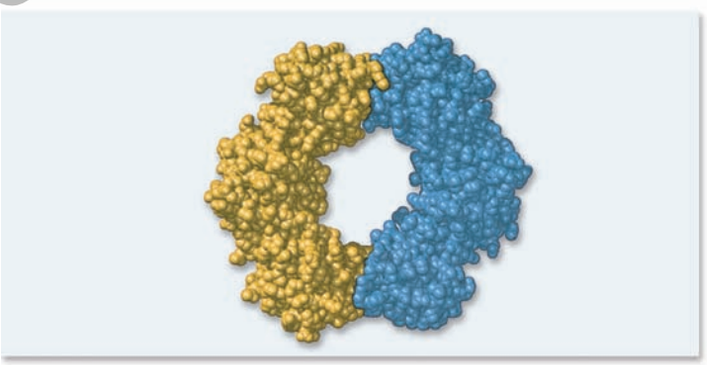
Lagging-strand synthesis

The discontinuous nature of synthesis on the lagging strand requires the cell to do much more work than on the leading strand (see figure 14.15). Primase is needed to synthesize primers for each Okazaki fragment, and then all these RNA primers need to be removed and replaced with DNA. Finally, the fragments need to be stitched together.

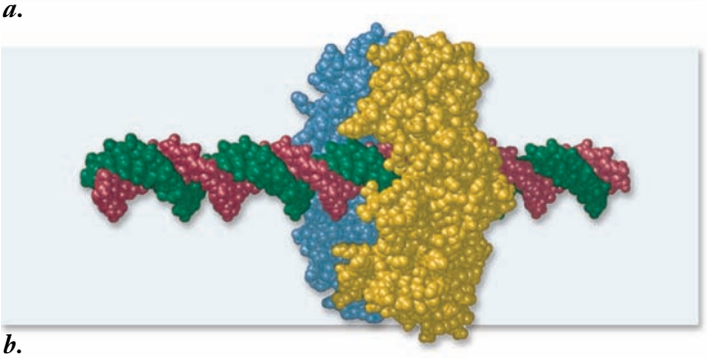
DNA Pol II accomplishes the synthesis of Okazaki fragments. The removal and replacement of primer segments, however, is accomplished by DNA Pol I. Using its 5′-to-3′ exonuclease activity, it can remove primers in front and then replace them by using its usual 5′-to-3′ polymerase activity. The synthesis is primed by the previous Okazaki fragment, which is composed of DNA and has a free 3′ OH that can be extended.

TABLE 14.1 DNA Replication Enzymes of *E. coli*

Protein	Role	Size (kDa)	Molecules per Cell
Helicase	Unwinds the double helix	300	20
Primase	Synthesizes RNA primers	60	50
Single-strand binding protein	Stabilizes single-stranded regions	74	300
DNA gyrase	Relieves torque	400	250
DNA polymerase III	Synthesizes DNA	≈900	20
DNA polymerase I	Erases primer and fills gaps	103	300
DNA ligase	Joins the ends of DNA segments; DNA repair	74	300



a.



b.

Figure 14.16 The DNA polymerase sliding clamp. ***a.*** The β subunit forms a ring that can encircle DNA. ***b.*** The β subunit is shown attached to the DNA. This forms the "sliding clamp" that keeps the polymerase attached to the template.

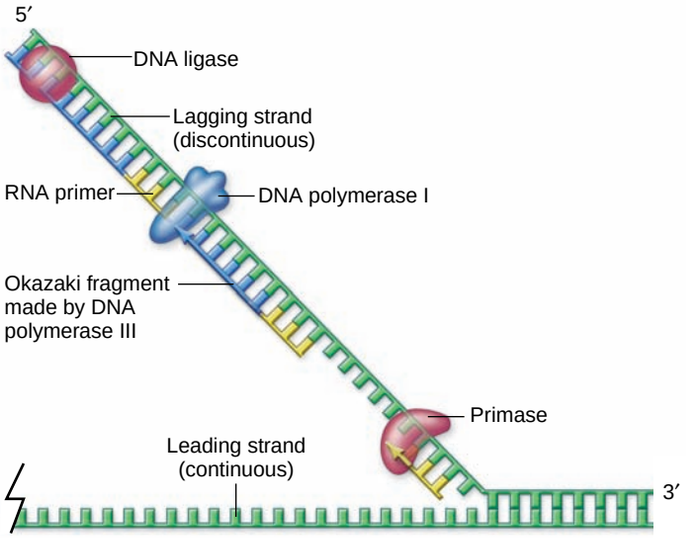


Figure 14.17 Lagging-strand synthesis. The action of primase synthesizes the primers needed by DNA polymerase III (not shown). These primers are removed by DNA polymerase I using its 5′-to-3′ exonuclease activity, then extending the previous Okazaki fragment to replace the RNA. The nick between Okazaki fragments after primer removal is sealed by DNA ligase.

This leaves only the last phosphodiester bond to be formed where synthesis by Pol I ends. This is done by **DNA ligase,** which seals this "nick," eventually joining the Okazaki fragments into complete strands. All of this activity on the lagging strand is summarized in figure 14.17.

Inquiry question

What is the role of DNA ligase? What would happen to DNA replication in a cell where this enzyme is not functional?

Termination

Termination occurs at a specific site located roughly opposite *oriC* on the circular chromosome. The last stages of replication produce two daughter molecules that are intertwined like two rings in a chain. These intertwined molecules are unlinked by the same enzyme that relieves torsional strain at the replication fork—DNA gyrase.

The replisome contains all the necessary enzymes for replication

The enzymes involved in DNA replication form a macromolecular assembly called the **replisome.** This assembly can be thought of as the "replication organelle," just as the ribosome is the organelle that synthesizes protein. The replisome has two main subcomponents: the *primosome*, and a complex of two DNA Pol III enzymes, one for each strand. The primosome is composed of primase and helicase, along with a number of accessory proteins. The need for constant priming on the lagging strand explains the need for the primosome complex as part of the replisome.

The two Pol III complexes include two synthetic core subunits, each with its own β subunit. The entire replisome complex is held together by a number of proteins that includes the clamp loader. The clamp loader is required to periodically load a β subunit on the lagging strand and to transfer the Pol III to this new β subunit (figure 14.18).

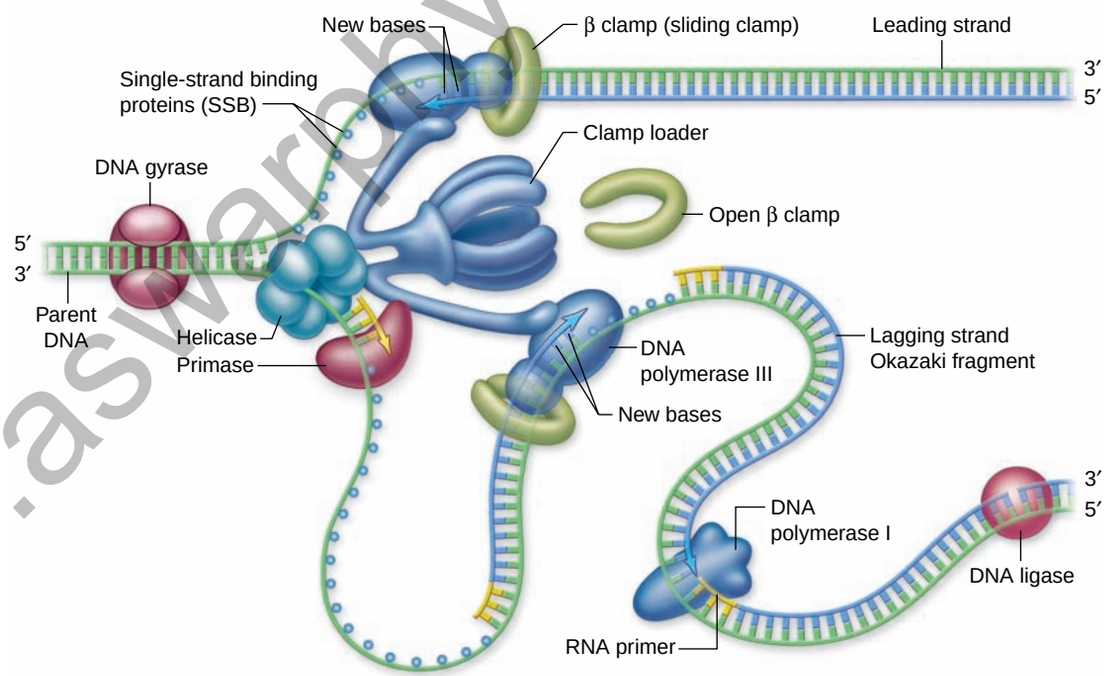


Figure 14.18 The replication fork. A model for the structure of the replication fork with two polymerase III enzymes held together by a large complex of accessory proteins. These include the "clamp loader," which loads the β subunit sliding clamp periodically on the lagging strand. The polymerase III on the lagging strand periodically releases its template and reassociates along with the β clamp. The loop in the lagging-strand template allows both polymerases to move in the same direction despite DNA being antiparallel. Primase, which makes primers for the lagging-strand fragments, and helicase are also associated with the central complex. Polymerase I removes primers and ligase joins the fragments together.