20 Sexual Reproduction, Meiosis, and Genetic Recombination

Mitotic cell division, which we discussed in the preceding chapter, is used for the proliferation of most eukaryotic cells, leading to the production of more organisms or more cells per organism. Since a mitotic cell division cycle involves one round of DNA replication followed by the segregation of identical chromatids into two daughter cells, mitotic division produces cells that are genetically identical, or very nearly so. This ability to perpetuate genetic traits faithfully allows mitotic division to form the basis of **asexual reproduction** *in eukaryotes. During asexual reproduction, new individuals are generated by mitotic division of cells in a single parent organism, either unicellular or multicellular. Although the details vary among organisms, asexual reproduction is widespread in nature. Examples include* mitotic division *of unicellular organisms,* budding *of offspring from a multicellular parent's body, and* regeneration *of whole organisms from pieces of a parent organism. In plants, entire organisms can even be regenerated from single cells taken from an adult plant.*

Asexual reproduction can be an efficient and evolutionarily successful mode of perpetuating a species. As long as environmental conditions remain essentially constant, the genetic predictability of asexual reproduction is perfectly suited for maintaining the survival of a population. But if the environment changes, a population that reproduces asexually may not be able to adapt to the new conditions. Under such conditions, organisms that reproduce sexually rather than asexually will usually have an advantage, as we now discuss.

Sexual Reproduction

In contrast to asexual reproduction, in which progeny are genetically identical to the single parent from which they arise, **sexual reproduction** allows genetic information from two parents to be mixed together, thereby producing offspring that are genetically dissimilar, both from each other and from the parents. Moreover, the offspring are unpredictably dissimilar; that is, we cannot anticipate exactly which combination of genes a particular offspring will receive from its two parents. Since most plants and animals—and even many eukaryotic microorganisms—reproduce sexually, this type of reproduction must provide some distinct advantages.

Sexual Reproduction Produces Genetic Variety by Bringing Together Chromosomes from Two Different Parents

Sexual reproduction allows genetic traits found in different individuals to be combined in various ways in newly developing offspring, thereby generating enormous variety among the individuals that make up a population. Genetic variation ultimately depends on the occurrence of *mutations,* which are unpredictable alterations in DNA base sequence. Mutations are rare events, and beneficial mutations are even rarer. But when beneficial mutations do arise, it is clearly advantageous to preserve them in the population. It can be even more beneficial to bring mutations together in various combinations—and therein lies an advantage of sexual reproduction. Although mutations occur in both sexual and asexual species, only sexual reproduction can bring about a reshuffling of genetic information in each new offspring.

Because sexual reproduction combines genetic information from two different parents into a single offspring, at some point in its life cycle every sexually reproducing organism has cells that contain two copies of each type of chromosome, one inherited from each parent. The two members of each chromosome pair are called **homologous chromosomes.** Two homologous chromosomes carry the same lineup of genes, although for any given gene, the two versions may differ slightly in base sequence. Not surprisingly, homologous chromosomes

usually look alike when viewed with a microscope (see Figure 19-23). An exception to this rule is the **sex chromosomes,** which determine whether an individual is male or female. The two kinds of sex chromosomes, generally called X and Y chromosomes, differ significantly in genetic makeup and appearance. In mammals, for example, females have two X chromosomes of the same size, whereas males have one X chromosome and a Y chromosome that is much smaller. Nonetheless, parts of the X and Y chromosomes are actually homologous; and, during sexual reproduction, the X and Y chromosomes behave as homologues.

A cell or organism with two sets of chromosomes is said to be **diploid** (from the Greek word *diplous,* meaning "double") and contains two copies of its genome. A cell or organism with a single set of chromosomes, and therefore a single copy of its genome, is **haploid** (from the Greek word *haplous,* meaning "single"). By convention, the haploid chromosome number for a species is designated n (or $1n$) and the diploid number $2n$. For example, in humans $n = 23$, which means that most human cells contain two sets of 23 chromosomes, yielding a diploid total of 46. The diploid state is an essential feature of the life cycle of sexually reproducing species. In a sense, a diploid cell contains an extra set of genes that is available for mutation and genetic innovation. Changes in a second copy of a gene usually will not threaten the survival of an organism, even if the mutation disrupts the original function of that particular gene copy. In addition, the diploid state provides some protection against chromosome damage; if one chromosome is accidentally broken, it can sometimes be repaired using the DNA sequence of the homologous chromosome as a template (page 626).

Diploid Cells May Be Homozygous or Heterozygous for Each Gene

To further explore the genetic consequences of the diploid state, let's now focus on the behavior of an individual **gene locus** (plural: **loci**), which is the place on a chromosome that contains the DNA sequence for a particular gene. For simplicity, we will assume that the gene controls a single, clear-cut characteristic—or *character,* as geneticists usually say—in the organism. Let's also assume that only one copy of this gene is present per haploid genome, so that a diploid organism will have two copies of the gene, which may be either identical or slightly different. The two versions of the gene are called **alleles,** and the combination of alleles determines how an organism will express the character controlled by the gene. In garden peas, for example, the alleles at one particular locus determine seed color, which may be green or yellow (**Figure 20-1**). An organism with two identical alleles for a given gene is said to be **homozygous** for that gene or character. Thus, a pea plant that inherited the same allele for yellow seed color from both of its parents is said to be homozygous for seed color. An organism with two different alleles for a gene is

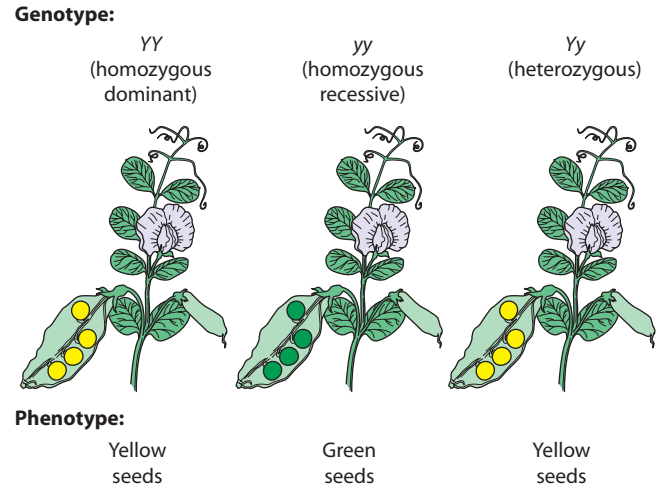


FIGURE 20-1 Genotype and Phenotype. In garden peas, seed color (phenotype) can be either yellow or green. The seed-color alleles are *Y* (yellow, dominant) or *y* (green, recessive). Because the pea plant is a diploid organism, its genetic makeup (genotype) for seed color may be homozygous dominant *(YY)*, homozygous recessive *(yy)*, or heterozygous *(Yy)*.

said to be **heterozygous** for that gene or for the character it determines. A pea plant with one allele that specifies yellow seed color and a second allele that specifies green seed color is therefore heterozygous for seed color.

In a heterozygous individual, one of the two alleles is often **dominant** and the other **recessive.** These terms convey the idea that the dominant allele determines how the trait will appear in a heterozygous individual. (The word *trait* refers to a particular variant of a character, such as green or yellow seeds, where seed color is the character.) For seed color in peas, the yellow trait is dominant over green; this means that pea plants heterozygous for seed color have yellow seeds. A dominant allele is usually designated by an uppercase letter that stands for the trait, whereas a corresponding recessive allele is represented by the same letter in lowercase. In both cases, italics are used. Thus, alleles for seed color in peas are represented by *Y* for yellow and *y* for green because the yellow trait is dominant. As Figure 20-1 illustrates, a pea plant can be homozygous for the dominant allele *(YY)*, homozygous for the recessive allele *(yy)*, or heterozygous *(Yy)*.

It is important to distinguish between the **genotype,** or genetic makeup of an organism, and its **phenotype,** or the physical expression of its genotype. The phenotype of an organism can usually be determined by inspection (e.g., by looking to see whether seeds are green or yellow). Genotype, on the other hand, can be *directly* determined only by studying an organism's DNA. The genotype also can be deduced from indirect evidence, such as the organism's phenotype and information about the phenotypes of its parents and/or offspring. Organisms exhibiting the same phenotype do not necessarily have identical genotypes. In the example of Figure 20-1, pea plants with

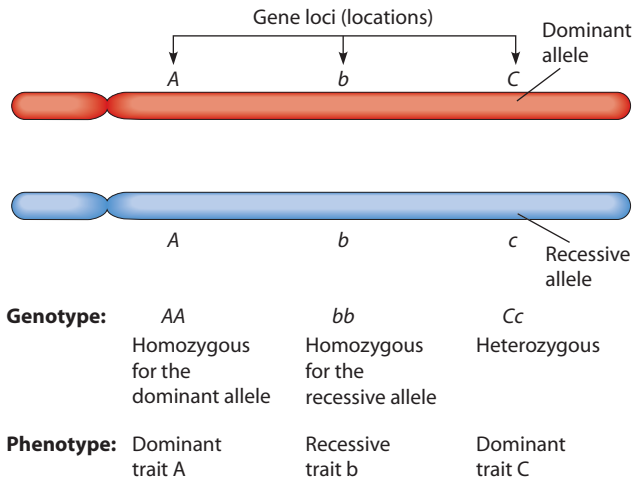


FIGURE 20-2 Some Genetic Terminology. This diagram shows a homologous pair of chromosomes from a diploid cell; the chromosomes are the same size and shape and carry genes for the same characters (characteristics), in the same order. The site of a gene on a chromosome is called a gene locus. The particular versions of a gene—alleles—found at comparable gene loci on homologous chromosomes may be identical (giving the organism a genotype that is homozygous for that gene) or different (making the genotype heterozygous). If one allele of a gene is dominant and the other recessive, the heterozygous organism exhibits a dominant phenotype with respect to the character in question. A recessive phenotypic trait is observed only if the genotype is homozygous for the recessive allele. Red and blue are used here and in most later figures to distinguish the different parental origins of the two chromosomes.

yellow seeds (phenotype) can be either *YY* or *Yy* (genotype). **Figure 20-2** summarizes the genetic terminology introduced so far.

Gametes Are Haploid Cells Specialized for Sexual Reproduction

The hallmark of sexual reproduction is that genetic information contributed by two parents is brought together in a single individual. Because the offspring of sexual reproduction are diploid, the contribution from each parent must be haploid. The haploid cells produced by each parent that fuse together to form the diploid offspring are called **gametes,** and the process that produces them is **gametogenesis.** Biologists distinguish between male and female individuals on the basis of the gametes they produce. Gametes produced by males, called **sperm** (or *spermatozoa*), are usually quite small and may be inherently motile. Female gametes, called **eggs** or **ova** (singular: **ovum**), are specialized for the storage of nutrients and tend to be quite large and nonmotile. For example, in sea urchins the volume of an egg cell is more than 10,000 times greater than that of a sperm cell; in birds and amphibians, which have massive yolky eggs, the size difference is even greater. But despite their differing sizes, sperm and egg bring equal amounts of chromosomal DNA to the offspring.

The union of sperm and egg during sexual reproduction is called **fertilization.** The resulting fertilized egg, or **zygote,** is diploid, having received one chromosome set from the sperm and a homologous set from the egg. In the life cycles of multicellular organisms, fertilization is followed by a series of mitotic divisions and progressive specialization of various groups of cells to form a multicellular embryo and eventually an adult.

In a few unusual situations, eggs can develop into offspring without the need for sperm—a phenomenon known as *parthenogenesis.* A striking case in point is the Komodo dragon, an inhabitant of the central islands of Indonesia. The Komodo dragon is the world's largest species of lizard, growing to an average length of 7–10 feet. When male Komodos are unavailable for sexual reproduction, female Komodos give birth to offspring through a self-fertilization process involving the fusion of a haploid egg cell with a haploid *polar body*—a "mini-egg" produced at the same time eggs are formed (see page 611). Consequently, the genes of each offspring derive solely from the mother, though the offspring are not exact duplicates of the mother because different combinations of alleles are present in the haploid cells that fuse together to create the embryo.

In some organisms, gametes cannot be categorized as being male or female. Certain fungi and unicellular eukaryotes, for example, produce gametes that are identical in appearance but differ slightly at the molecular level. Such gametes are said to differ in **mating type.** The union of two of these gametes requires that they be of different mating types, but the number of possible mating types in a species may be greater than two—in some cases, more than ten!

Meiosis

Since gametes are haploid, they cannot be produced from diploid cells by mitosis because mitosis creates daughter cells that are genetically identical to the original parent cell. In other words, if gametes were formed by mitotic division of diploid cells, both sperm and egg would have a diploid chromosome number, just like the parent diploid cells. The hypothetical zygote created by the fusion of such diploid gametes would be *tetraploid* (i.e., possess *four* homologous sets of chromosomes). Moreover, the chromosome number would continue to double for each succeeding generation—an impossible scenario. Thus, for the chromosome number to remain constant from generation to generation, a different type of cell division must occur during the formation of gametes. That special type of division, called **meiosis,** reduces the chromosome number from diploid to haploid.

Meiosis involves one round of chromosomal DNA replication followed by two successive nuclear divisions. This results in the formation of four daughter nuclei (usually in separate daughter cells) containing one

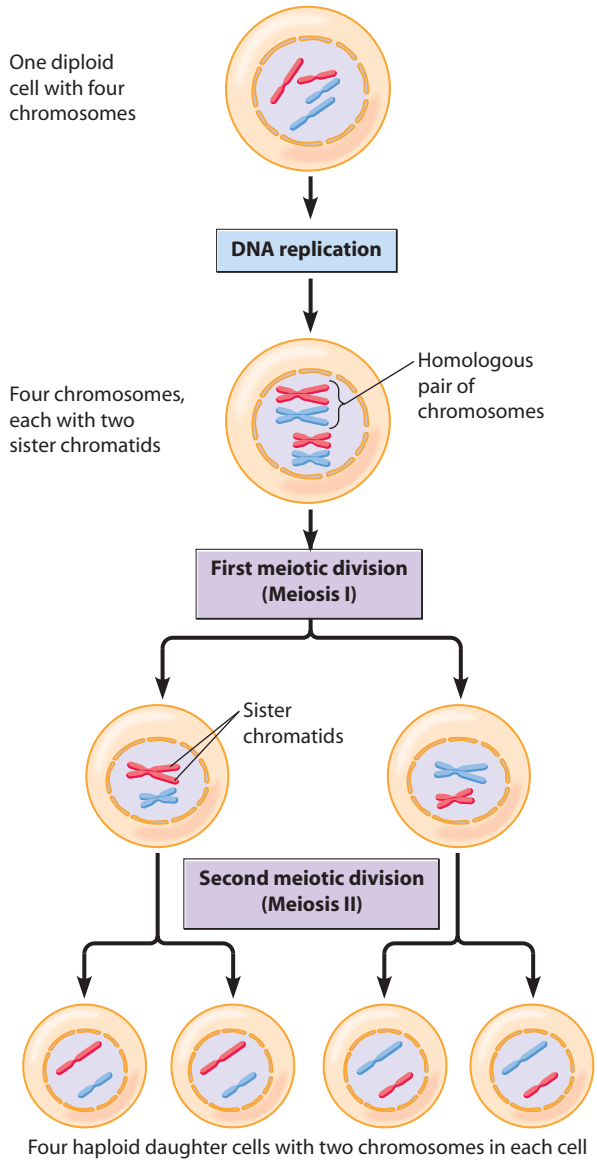


FIGURE 20-3 The Principle of Meiosis. Meiosis involves a single round of DNA replication (chromosome duplication) in a diploid cell followed by two successive cell division events. In this example, the diploid cell has only four chromosomes, which can be grouped into two homologous pairs. After DNA replication, each chromosome consists of two sister chromatids. In the first meiotic division (meiosis I), homologous chromosomes separate, but sister chromatids remain attached. In the second meiotic division (meiosis II), sister chromatids separate, resulting in four haploid daughter cells with two chromosomes each. Notice that each haploid cell has one chromosome from each homologous pair that was present in the diploid cell. For simplicity, the effects of crossing over and genetic recombination are not shown in this diagram.

ACTIVITIES www.thecellplace.com *Meiosis*

haploid set of chromosomes per nucleus. **Figure 20-3** outlines the principle of meiosis starting with a diploid cell containing four chromosomes ($2n = 4$). A single round of DNA replication is followed by two cell divisions, meiosis I and meiosis II, leading to the formation of four haploid cells.

The Life Cycles of Sexual Organisms Have Diploid and Haploid Phases

Meiosis and fertilization are indispensable components of the life cycle of every sexually reproducing organism, because the doubling of chromosome number that takes place at fertilization is balanced by the halving that occurs during meiosis. As a result, the life cycle of sexually reproducing organisms is divided into two phases: a diploid ($2n$) phase and a haploid ($1n$) phase. The diploid phase begins at fertilization and extends until meiosis, whereas the haploid phase is initiated at meiosis and ends with fertilization.

Organisms vary greatly in the relative prominence of the haploid and diploid phases of their life cycles, as shown for some representative groups in **Figure 20-4**. Some fungi are examples of sexually reproducing organisms whose life cycles are primarily haploid but include a brief diploid phase that begins with gamete fusion (the fungal equivalent of fertilization) and ends with meiosis (Figure 20-4b). Meiosis usually takes place almost immediately after gamete fusion, so the diploid phase is very short. Accordingly, only a very small fraction of nuclei in such fungi are diploid at any one time. Fungal gametes develop, without meiosis, from cells that are already haploid.

Mosses and ferns are probably the best examples of organisms in which both the haploid and diploid phases are prominent features of the life cycle. Every species of these plants has two alternative, morphologically distinct, multicellular forms, one haploid and the other diploid (Figure 20-4c). For mosses, the haploid form of the organism is larger and more prominent, and the diploid form is smaller and more short-lived. For ferns, it is the other way around. In both cases, gametes develop from preexisting haploid cells.

Organisms that alternate between haploid and diploid multicellular forms in this way are said to display an **alternation of generations** in their life cycles. In addition to mosses and ferns, eukaryotic algae and other plants exhibit an alternation of diploid and haploid generations. In all such organisms, the products of meiosis are **haploid spores,** which, after germination, give rise by mitotic cell division to the haploid form of the plant or alga. The haploid form in turn produces the gametes by specialization of cells that are already haploid. The gametes, upon fertilization, give rise to the diploid form. Because the diploid form produces spores, it is called a **sporophyte** ("spore-producing plant"). The haploid form produces gametes and is therefore called a **gametophyte.** While all plants exhibit an alternation of generations, in most cases the sporophyte generation predominates. In flowering plants, for example, the gametophyte generation is an almost vestigial structure located in the flower (female gametophyte in the *carpel,* male gametophytes in the flower's *anthers*).

The best examples of life cycles dominated by the diploid phase are found in animals (Figure 20-4d). In such

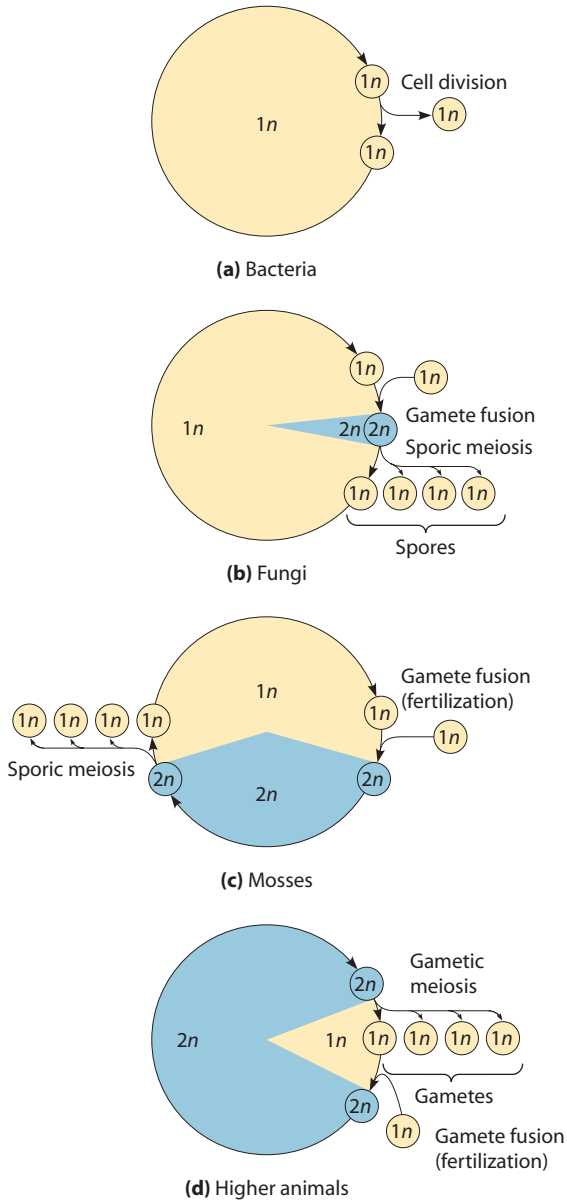


FIGURE 20-4 Types of Life Cycles. The relative prominence of the haploid ($1n$) and diploid ($2n$) phases of the life cycle differ greatly, depending on the organism. **(a)** Bacteria exist exclusively in the haploid state. **(b)** Many fungi exemplify a life form that is predominantly haploid but has a brief diploid phase. Because the products of meiosis in fungi are haploid spores, this type of meiosis is called sporic meiosis. The spores give rise to haploid cells, some of which later become gametes (without meiosis). **(c)** Mosses (and ferns as well) alternate between haploid and diploid forms, both of which are significant components in the life cycles of these organisms. Sporic meiosis produces haploid spores, which in this case grow into haploid plants. Eventually, some of the haploid plant's cells differentiate into gametes. (In seed plants, such as conifers and flowering plants, the haploid forms of the organism are vestigial, each consisting of only a small number of cells.) **(d)** Higher animals are the best examples of organisms that are predominantly diploid, with only the gametes representing the haploid phase of the life cycle. Animals are said to have a gametic meiosis, since the immediate products of meiosis are haploid gametes.

organisms, including humans, meiosis gives rise not to spores but to gametes directly, so the haploid phase of the life cycle is represented only by the gametes. Meiosis in such species is called *gametic meiosis* to distinguish it from the *sporic meiosis* observed in spore-producing organisms exhibiting an alternation of generations. Meiosis is thus gametic in animals and sporic in plants.

Meiosis Converts One Diploid Cell into Four Haploid Cells

Wherever it occurs in an organism's life cycle, meiosis is always preceded by chromosome duplication in a diploid cell and involves two successive divisions that convert the diploid nucleus into four haploid nuclei. **Figure 20-5** illustrates the various phases of meiosis; refer to it as you read the following discussion.

The first meiotic division, or **meiosis I,** is sometimes referred to as the *reduction division* of meiosis because it is the event that reduces the chromosome number from diploid to haploid. Early during meiosis I, the two chromosomes of each homologous pair come together during prophase to exchange some of their genetic information (using a mechanism to be discussed shortly). This pairing of homologous chromosomes, called **synapsis,** is unique to meiosis; at all other times, including mitosis, the chromosomes of a homologous pair behave independently.

The two chromosomes of each homologous pair bind together so tightly during the first meiotic prophase that they behave as a single unit called a **bivalent** (or *tetrad,* which emphasizes that each of the two homologous chromosomes consists of two sister chromatids, yielding a total of four chromatids). After aligning at the spindle equator, each bivalent splits apart in such a way that its two homologous chromosomes move to opposite spindle poles. Because each pole receives only one member of each homologous pair, the daughter nuclei produced by meiosis I are considered to be haploid (even though the individual chromosomes in these nuclei are composed of two sister chromatids). During the second meiotic division (meiosis II), which closely resembles a mitotic division, the two sister chromatids of each chromosome separate into two daughter cells. Hence, the events unique to meiosis happen during the first meiotic division: the synapsis of homologous chromosomes and their subsequent segregation into different daughter nuclei.

Meiotic cell divisions involve the same basic stages as mitosis, although cell biologists do not usually distinguish prometaphase as a separate phase. Thus, the meiotic phases are *prophase, metaphase, anaphase,* and *telophase.* We will see shortly that prophase I is much longer and more complicated than mitotic prophase, whereas prophase II tends to be quite short. Another important difference from mitosis is that a normal interphase does not intervene between the two meiotic divisions. If an interphase does take place, it is usually very short and—most

importantly—does *not* include DNA replication, because each chromosome already consists of a pair of replicated sister chromatids that had been generated prior to the first meiotic division. The purpose of the second meiotic division, like that of a typical mitotic division, is to parcel these sister chromatids into two daughter nuclei.

Meiosis I Produces Two Haploid Cells That Have Chromosomes Composed of Sister Chromatids

The first meiotic division segregates homologous chromosomes into different daughter cells. This feature of meiosis has special genetic significance because it represents the point in an organism's life cycle when the two alleles for each gene part company. And it is this separation of alleles that makes possible the eventual remixing of different pairs of alleles at fertilization. Also of great significance during the first meiotic division are events involving the physical exchange of parts of DNA molecules. Such an exchange of DNA segments between two different sources is called **genetic recombination** by molecular biologists. As we will discuss shortly, this type of DNA exchange between homologous chromosomes takes place when the chromosomes are synapsed during prophase I.

Prophase I: Homologous Chromosomes Become Paired and Exchange DNA. Prophase I is a particularly long and complex phase. Based on light microscopic observations, early cell biologists divided prophase I into five stages called *leptotene, zygotene, pachytene, diplotene,* and *diakinesis* (**Figure 20-6**, p. 608).

The **leptotene** stage begins with the condensation of chromatin fibers into long, threadlike structures, similar to what occurs at the beginning of mitosis. At **zygotene,** continued condensation makes individual chromosomes distinguishable, and homologous chromosomes become closely paired with each other via the process of synapsis, forming bivalents. Keep in mind that each bivalent has four chromatids, two derived from each chromosome. Bivalent formation is of considerable genetic significance because the close proximity between homologous chromosomes allows DNA segments to be exchanged by a process called **crossing over.** It is this physical exchange of genetic information between corresponding regions of homologous chromosomes that accounts for genetic recombination. You will learn more about the molecular nature of crossing over later in this chapter. Crossing over occurs during the **pachytene** stage, which is marked by a dramatic compacting process that reduces each chromosome to less than a quarter of its previous length.

At the **diplotene** stage, the homologous chromosomes of each bivalent begin to separate from each other, particularly near the centromere. However, the two chromosomes of each homologous pair remain attached by connections known as **chiasmata** (singular: **chiasma**). Such connections are situated in regions where homologous chromosomes have exchanged DNA segments and hence provide visual evidence that crossing over has occurred between two chromatids, one derived from each chromosome.

In some organisms—female mammals, for instance—the chromosomes decondense during diplotene, transcription resumes, and the cells "take a break" from meiosis for a prolonged period of growth, sometimes lasting for years. (We will consider this situation at the end of our discussion of meiosis.) With the onset of **diakinesis,** the final stage of prophase I, the chromosomes recondense to their maximally compacted state. Now the centromeres of the homologous chromosomes separate further, and the chiasmata eventually become the only remaining attachments between the homologues. At this stage, the nucleoli disappear, the spindle forms, and the nuclear envelope breaks down, marking the end of prophase I.

With the advent of modern tools, especially the electron microscope, cell biologists have been able to refine our picture of what happens during prophase I. They have found that what holds homologous chromosomes in tight apposition during synapsis is the **synaptonemal complex,** an elaborate protein structure resembling a zipper (**Figure 20-7**, p. 609). The *lateral elements* of the synaptonemal complex start to attach to individual chromosomes during leptotene, but the *central element,* which actually joins homologous chromosomes together, does not form until zygotene (see Figure 20-7b). How do the members of each pair of homologous chromosomes find each other so they can be joined by a synaptonemal complex? During early zygotene, the ends (telomeres) of each chromosome become clustered on one side of the nucleus and attach to the nuclear envelope, with the body of each chromosome looping out into the nucleus. To picture this, imagine holding all the ends of four ropes (two long and two short) together. If you give the ropes a strong shake, they will settle into four loops, arranged according to length. This type of chromosome configuration, called a *bouquet,* is thought to promote chromosome alignment.

The alignment of similar-sized chromosomes facilitates formation of synaptonemal complexes, which become fully developed during pachytene. Formation of synaptonemal complexes is closely associated with the process of crossing over in higher eukaryotes, and some electron micrographs reveal additional protein complexes, called *recombination nodules,* that may mediate the crossing over process. The synaptonemal complexes then disassemble during diplotene, allowing the homologous chromosomes to separate (except where they are joined by chiasmata).

Metaphase I: Bivalents Align at the Spindle Equator. During metaphase I, the bivalents attach via their kinetochores to spindle microtubules and migrate to the spindle equator. The presence of *paired* homologous chromosomes (i.e., bivalents) at the spindle equator during metaphase I is a crucial difference between meiosis I and a

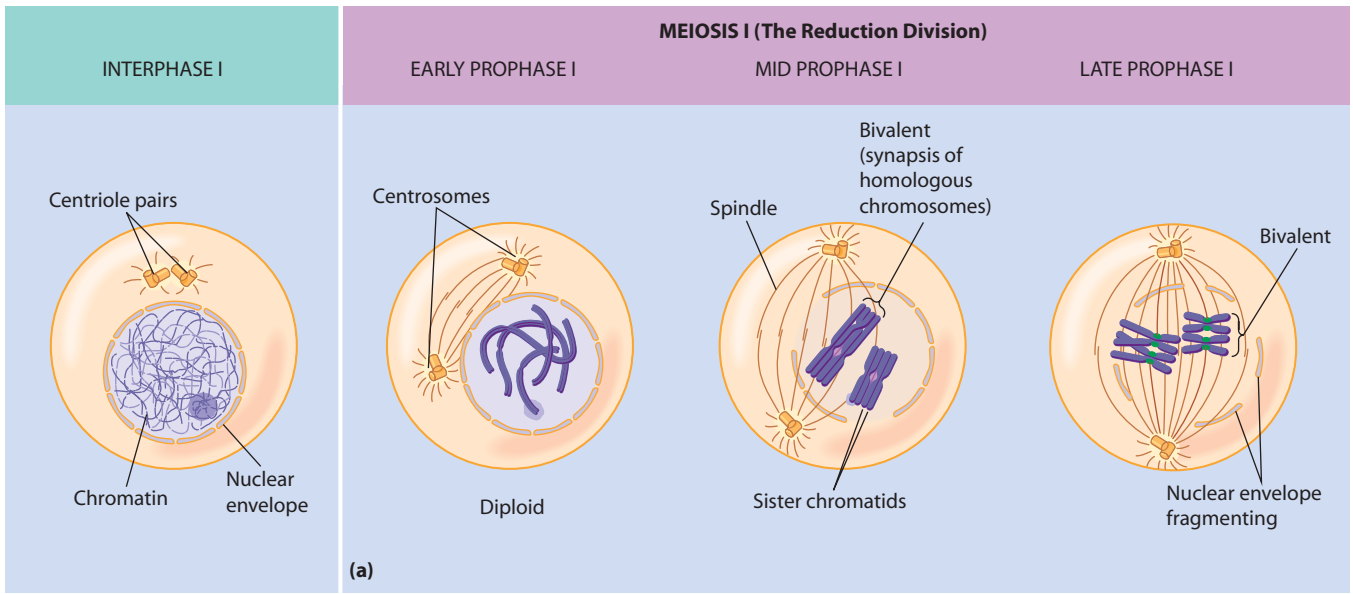


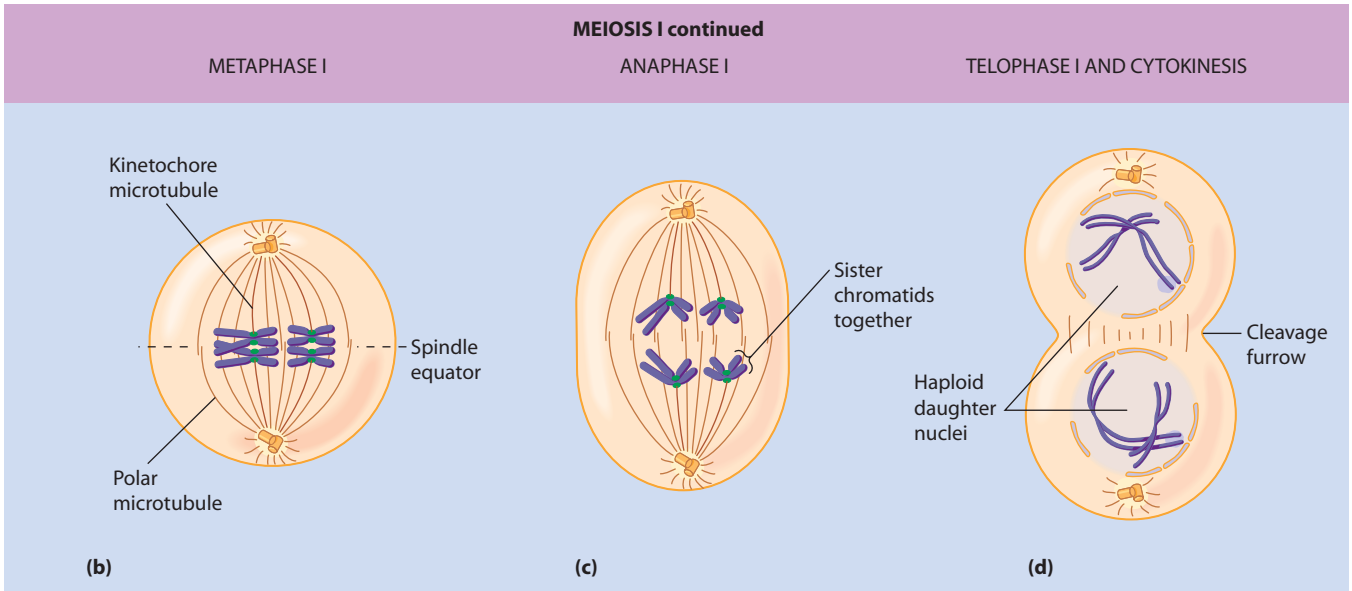
FIGURE 20-5 Meiosis in an Animal Cell. Meiosis consists of two successive divisions, called meiosis I and II, with no intervening DNA synthesis or chromosome duplication. **(a)** During prophase I, the chromosomes (duplicated during the previous S phase) condense and the two centrosomes migrate to opposite poles of the cell. Each chromosome (four in this example) consists of two sister chromatids. Homologous chromosomes pair to form bivalents. **(b)** Bivalents become aligned at the spindle equator (metaphase I). **(c)** Homologous chromosomes separate during anaphase I, but sister chromatids remain attached at the centromere. **(d)** Telophase and cytokinesis follow. Although not illustrated here, there may then be a short interphase (interphase II). In meiosis II, **(e)** chromosomes recondense (prophase II), **(f)** chromosomes align at the spindle equator (metaphase II), and **(g)** sister chromatids at last separate (anaphase II). **(h)** After telophase II and cytokinesis, the result is four haploid daughter cells, each containing one chromosome of each homologous pair. Prophase I is a complicated process shown in more detail in Figure 20-6. Meiosis in plants is similar, except for the absence of centrioles and the mechanism of cytokinesis, which involves formation of a cell plate (described in Chapter 19).

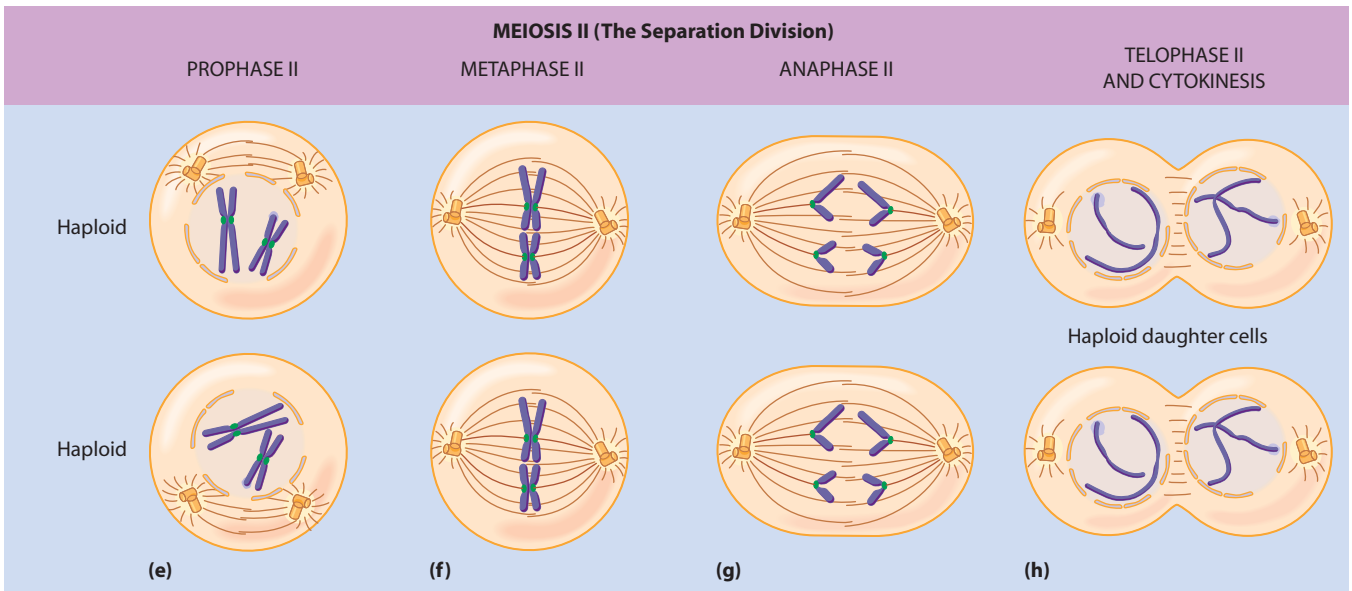
typical mitotic division, where such pairing is not observed (**Figure 20-8**, p. 610). Because each bivalent contains four chromatids (two sister chromatids from each chromosome), four kinetochores are also present. The kinetochores of sister chromatids lie side by side—in many species appearing as a single mass—and face the same pole of the cell. Such an arrangement allows the kinetochores derived from the sister chromatids of one homologous chromosome to attach to microtubules emanating from one spindle pole and the kinetochores derived from the sister chromatids of the other homologous chromosome to attach to microtubules emanating from the opposite spindle pole. This orientation sets the stage for separation of the homologous chromosomes during anaphase. The bivalents are randomly oriented at this point, in the sense that, for each bivalent, either the maternal or paternal homologue may face a given pole of the cell. As a result, each spindle pole (and hence each daughter cell) will receive a random mixture of maternal and paternal chromosomes when the two members of each chromosome pair move toward opposite spindle poles during anaphase.

At this stage, homologous chromosomes are held together solely by chiasmata. If for some reason prophase I had occurred without crossing over, and hence without chiasma formation, the chromosomes might not pair properly at the spindle equator, and homologous chromosomes might not separate properly during anaphase I. This is exactly what happens during meiosis in mutant yeast cells that exhibit deficiencies in genetic recombination.

Anaphase I: Homologous Chromosomes Move to Opposite Spindle Poles. At the beginning of anaphase I, the members of each pair of homologous chromosomes separate from each other and start migrating toward opposite spindle poles, pulled by their respective kinetochore microtubules. Again, note the fundamental difference between meiosis and mitosis (Figure 20-8). During mitotic anaphase, *sister chromatids separate* and move to opposite spindle poles, whereas in anaphase I of meiosis, *homologous chromosomes separate* and move to opposite spindle poles while sister chromatids remain together. Because the two members of each pair of homologous chromosomes move to opposite spindle poles during anaphase I, each pole receives a haploid set of chromosomes.

How can we explain the fact that sister chromatids separate from each other during mitosis but not during meiosis I? In Chapter 19, we saw that mitotic anaphase is associated with activation of the enzyme separase by the anaphase-promoting complex (see Figure 19-38); the activated separase in turn cleaves the cohesins that hold sister chromatids together, thereby allowing sister chromatids to separate. To prevent chromatid separation from taking





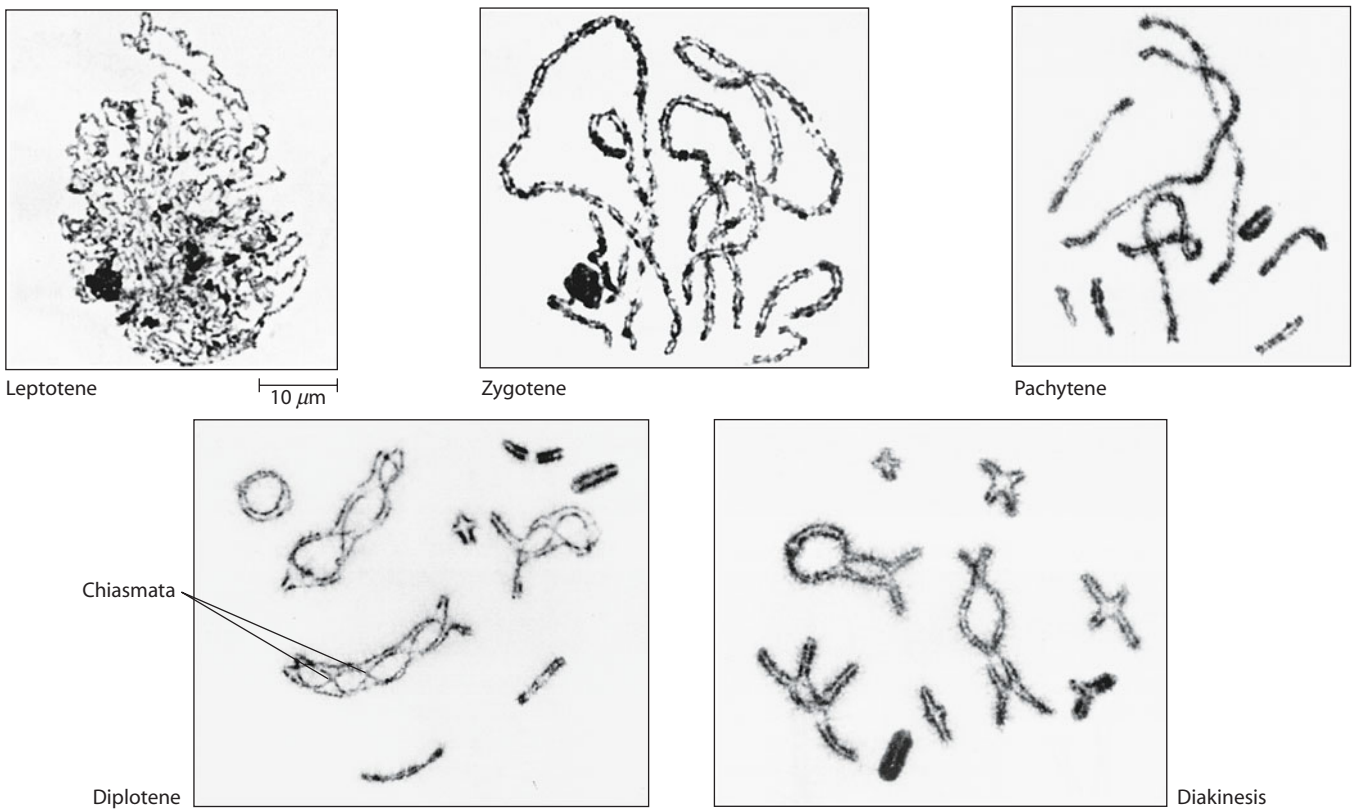
place during anaphase I of meiosis, a protein called *shugoshin* (Japanese for "guardian spirit") protects the cohesins located at the chromosomal centromere from being degraded by separase.

Telophase I and Cytokinesis: Two Haploid Cells Are Produced. The onset of telophase I is marked by the arrival of a haploid set of chromosomes at each spindle pole. Which member of a homologous pair ends up at which pole is determined entirely by how the chromosomes happened to be oriented at the spindle equator during metaphase I. After the chromosomes have arrived at the spindle poles, nuclear envelopes sometimes form around the chromosomes before cytokinesis ensues, generating two haploid cells whose chromosomes consist of sister chromatids. In most cases, the chromosomes do not decondense before meiosis II begins.

Meiosis II Resembles a Mitotic Division

After meiosis I has been completed, a brief interphase may intervene before **meiosis II** begins. However, this interphase is not accompanied by DNA replication because each chromosome already consists of a pair of replicated, sister chromatids that were generated by DNA synthesis during the interphase preceding meiosis I. *So DNA is replicated only once in conjunction with meiosis, and that is prior to the first meiotic division.* The purpose of meiosis II, like that of a typical mitotic division, is to parcel the sister chromatids created by this initial round of DNA replication into two newly forming cells. As a result, meiosis II is sometimes referred to as the *separation division* of meiosis.

Prophase II is very brief. If detectable at all, it is much like a mitotic prophase. Metaphase II also resembles the equivalent stage in mitosis, except that only half as many



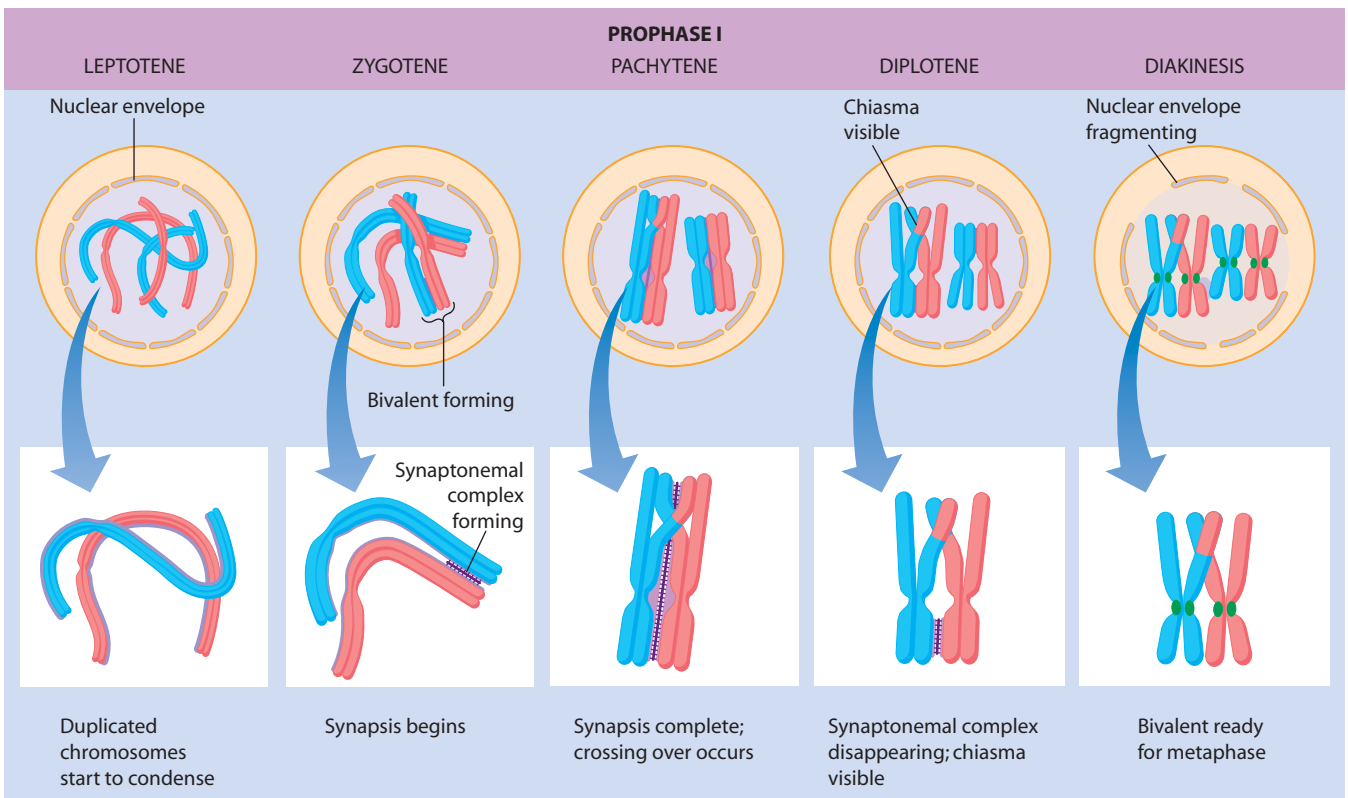


FIGURE 20-6 Meiotic Prophase I. Based on changes in chromosome behavior and appearance, prophase I is subdivided into the five stages shown in these photographs and schematic diagram. The diagram depicts the cell nucleus at each stage for a diploid cell containing four chromosomes (two homologous pairs). The lower part of the diagram focuses on a single homologous pair in greater detail, revealing the formation and subsequent disappearance of the synaptonemal complex, a protein structure that holds homologous chromosomes in close lateral apposition during pachytene. Red and blue distinguish the paternal and maternal chromosomes of each homologous pair; the synaptonemal complex is shown in shades of purple.

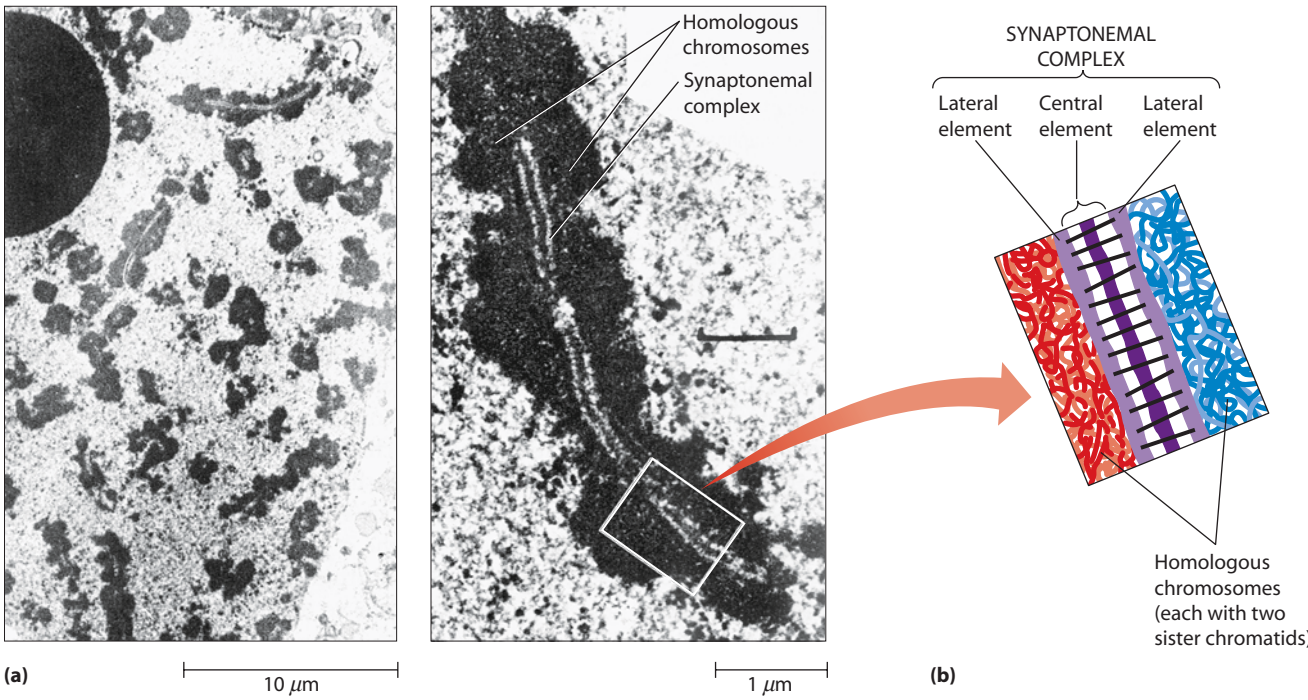


FIGURE 20-7 The Synaptonemal Complex. **(a)** These electron micrographs show, at two magnifications, synaptonemal complexes in the nuclei of cells from a lily. The cells are at the pachytene stage of prophase I. **(b)** The diagram identifies the complex's *lateral elements* (light purple), which seem to form on the chromosomes during leptotene, and its *central* (or *axial*) *element* (dark purple), which starts to appear during zygotene and "zips" the homologous chromosomes together. At pachytene, the homologues are held tightly together all along their lengths.

chromosomes are present at the spindle equator. The kinetochores of sister chromatids now face in opposite directions, allowing the sister chromatids to separate and move (as new daughter chromosomes) to opposite spindle poles during anaphase II. The remaining phases of the second meiotic division resemble the comparable stages of mitosis. The final result is the formation of four daughter cells, each containing a haploid set of chromosomes. Because the two members of each homologous chromosome pair were randomly distributed to the two cells produced by meiosis I, each of the haploid daughter cells produced by meiosis II contains a random mixture of maternal and paternal chromosomes. Moreover, each of these chromosomes is composed of a mixture of maternal and paternal DNA sequences created by crossing over during prophase I.

While each of the cells produced by meiosis normally contains a complete, haploid set of chromosomes, a rare malfunction called **nondisjunction** can produce cells that either lack a particular chromosome or contain an extra chromosome, a condition we termed *aneuploidy* in Chapter 19. Nondisjunction refers to the failure of homologous chromosomes (during anaphase I) or sister chromatids (during anaphase II) to separate from each other at the metaphase-anaphase transition. Instead, both chromosomes or chromatids remain together and move into one of the two daughter cells, thereby generating one cell containing both copies of the chromosome and one cell containing neither copy. The resulting gametes have an incorrect number of chromosomes and tend to produce defective embryos that die before birth. However, a few such gametes can participate in the formation of embryos that do survive. For example, if an abnormal human sperm containing two copies of chromosome 21 fertilizes a normal egg containing one copy of chromosome 21, the resulting embryo, possessing three copies of chromosome 21, can develop fully and lead to the birth of a live child. But this child will exhibit a series of developmental abnormalities—including short stature, broad hands, folds over the eyes, and low intelligence—that together constitute *Down syndrome.*

At this point, you may want to study Figure 20-8 in its entirety to review the similarities and differences between meiosis and mitosis. In this diagram, the amount of DNA present at various stages is indicated using the **C value,** which corresponds to the amount of DNA present in a single (haploid) set of chromosomes. In a diploid cell prior to S phase, the chromosome number is $2n$ and the DNA content is 2C because two sets of chromosomes are present. When DNA undergoes replication during S phase, the DNA content is doubled to 4C because each chromosome now consists of two chromatids. In meiosis I, segregation of homologous chromosomes into different daughter cells reduces the chromosome number from $2n$ to $1n$ and the DNA content from 4C to 2C. Sister chromatid separation during meiosis II then reduces the DNA

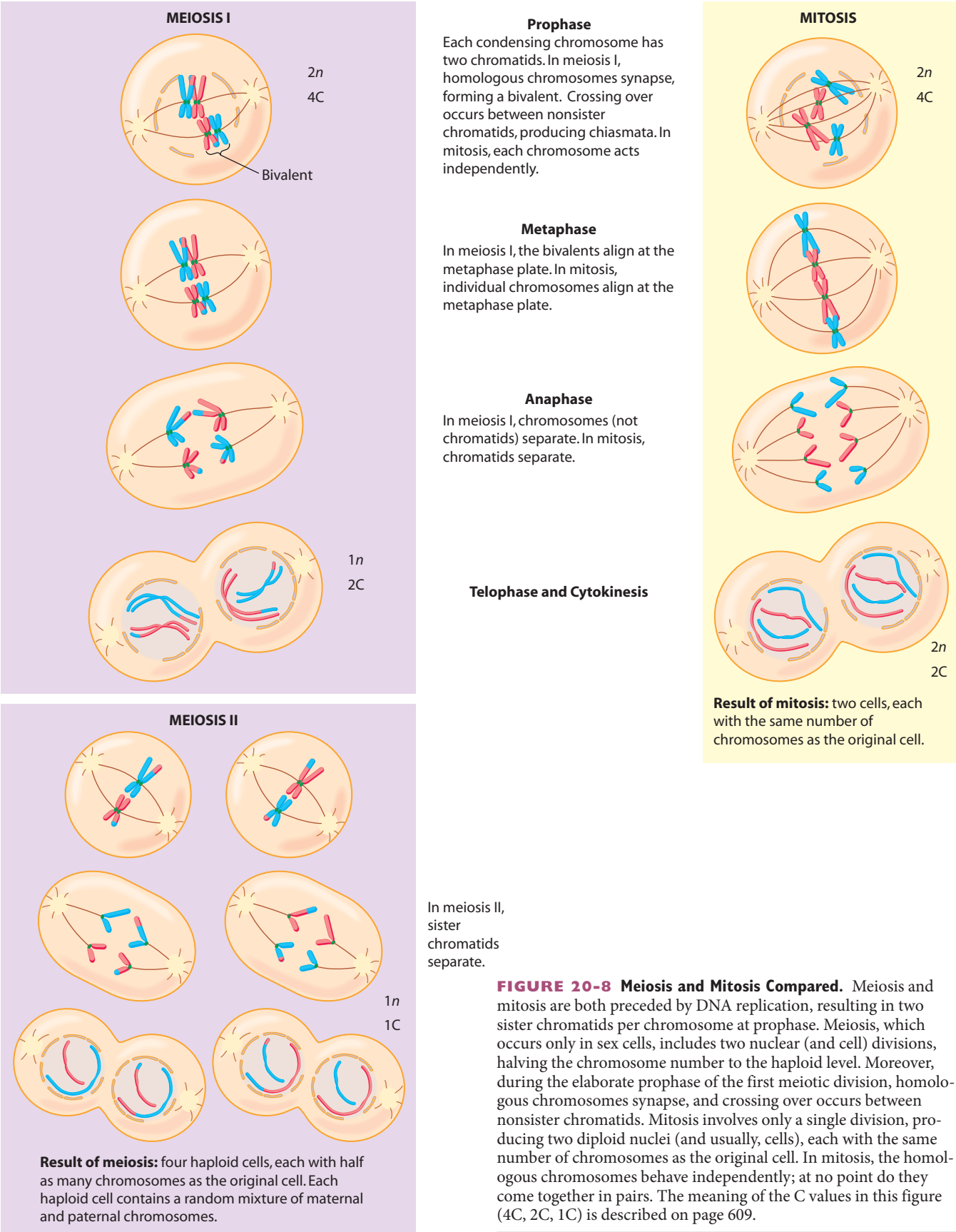


FIGURE 20-8 Meiosis and Mitosis Compared. Meiosis and mitosis are both preceded by DNA replication, resulting in two sister chromatids per chromosome at prophase. Meiosis, which occurs only in sex cells, includes two nuclear (and cell) divisions, halving the chromosome number to the haploid level. Moreover, during the elaborate prophase of the first meiotic division, homologous chromosomes synapse, and crossing over occurs between nonsister chromatids. Mitosis involves only a single division, producing two diploid nuclei (and usually, cells), each with the same number of chromosomes as the original cell. In mitosis, the homologous chromosomes behave independently; at no point do they come together in pairs. The meaning of the C values in this figure (4C, 2C, 1C) is described on page 609.

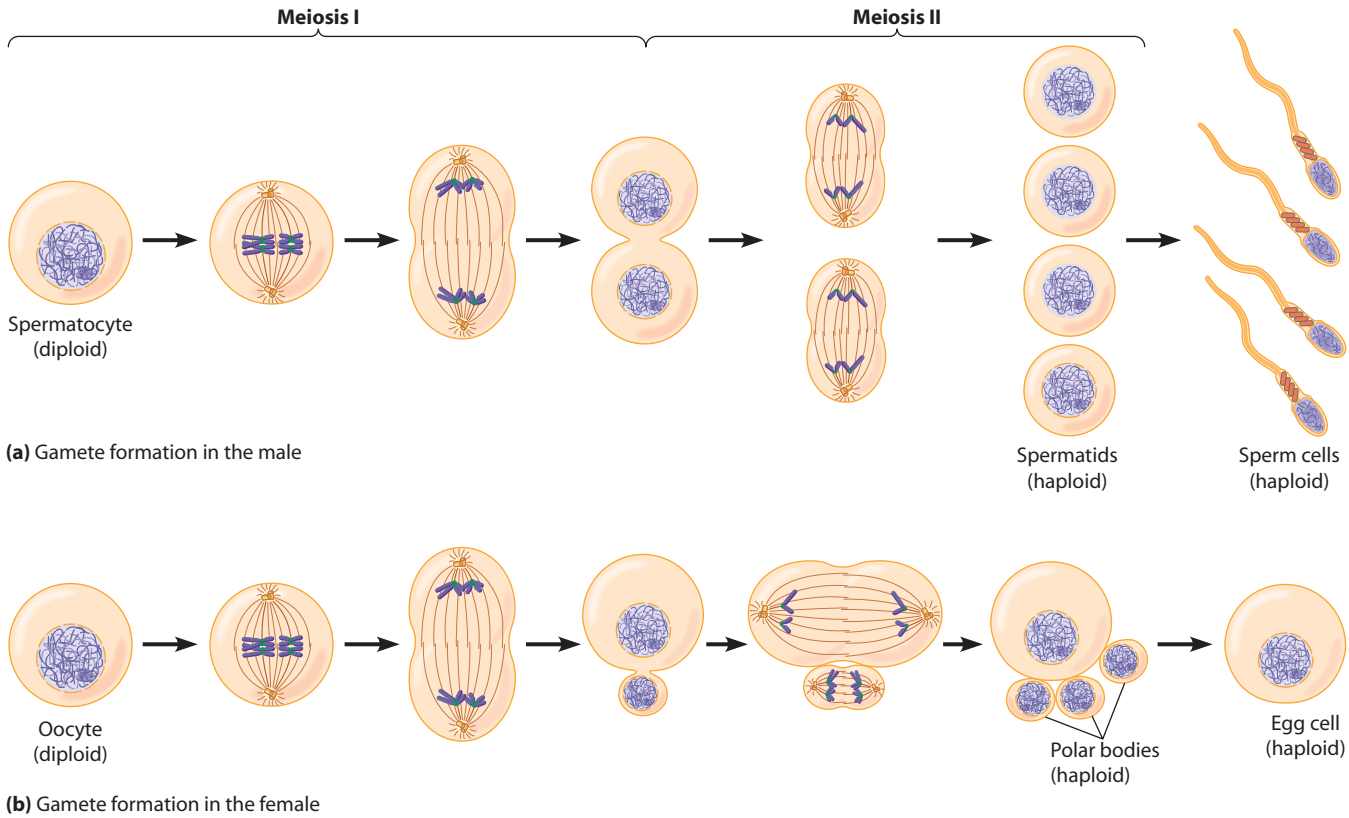


FIGURE 20-9 Gamete Formation. **(a)** In the male, all four haploid products of meiosis are retained and differentiate into sperm. **(b)** In the female, both meiotic divisions are asymmetric, forming one large egg cell and three (in some cases, only two) small cells called polar bodies that do not give rise to functional gametes. Although not indicated here, the mature egg cell usually grows much larger than the oocyte it arose from.

VIDEOS www.thecellplace.com *Meiosis I in sperm formation*

content from 2C to 1C, while the chromosome number remains at 1*n*. In contrast, a normal mitosis reduces the DNA content from 4C to 2C (by sister chromatid separation) while the chromosome number remains at 2*n*.

Sperm and Egg Cells Are Generated by Meiosis Accompanied by Cell Differentiation

Meiosis lies at the heart of gametogenesis, which as we saw earlier is the process of forming haploid gametes from diploid precursor cells. But male and female gametes differ significantly in structure, which means that gametogenesis must consist of more than just meiosis. **Figure 20-9** is a schematic depiction of gametogenesis in animals. In males, meiosis converts a diploid *spermatocyte* into four haploid *spermatids* of similar size (Figure 20-9a). After meiosis has been completed, the spermatids then differentiate into sperm cells by discarding most of their cytoplasm and developing flagella and other specialized structures.

In females, meiosis converts a diploid *oocyte* into four haploid cells, but only one of the four survives and gives rise to a functional egg cell (Figure 20-9b). This outcome is generated by two meiotic divisions that divide the cytoplasm of the oocyte unequally, with one of the four daughter cells receiving the bulk of the cytoplasm of the original diploid oocyte. The other three, smaller cells, called **polar bodies,** usually degenerate (although recall that the Komodo dragon can use a polar body to "fertilize" an egg). The advantage of having only one of the four haploid products of meiosis develop into a functional egg cell is that the cytoplasm that would otherwise have been distributed among four cells is instead concentrated into one egg cell, maximizing the content of stored nutrients in each egg.

An important difference between sperm and egg formation concerns the stage at which the cells acquire the specialized characteristics that make them functionally mature gametes. During sperm cell development, meiosis creates haploid spermatids that must then discard most of their cytoplasm and develop flagella before they are functionally mature. In contrast, developing egg cells acquire their specialized features *during* the process of meiosis. Many of the specialized features of the egg cell are acquired during prophase I, when meiosis is temporarily halted to allow time for extensive cell growth. During this *growth phase,* the cell also develops various types of external coatings designed to protect the egg from

chemical and physical injury. The amount of growth that takes place during this phase can be quite extensive. A human egg cell, for example, has a diameter of about 100 μm, giving it a volume more than a hundred times as large as that of the diploid oocyte from which it arose. And consider the gigantic size of a bird egg!

After the growth phase has been completed, developing oocytes remain arrested in prophase I until resumption of meiosis is initiated by an appropriate stimulus. In amphibians, resumption of meiosis is triggered by the steroid hormone progesterone, whose presence leads to an increase in the activity of the protein kinase, *MPF.* In Chapter 19, we showed that MPF is a Cdk-cyclin complex that controls mitotic cell division by triggering passage from G2 into M phase. MPF also controls meiosis by triggering the transition from prophase I to metaphase I. Progesterone exerts its control over MPF by stimulating the production of *Mos,* a protein kinase that activates a series of other protein kinases, which in turn leads to the activation of MPF.

In response to the activation of MPF, the first meiotic division is completed. In some organisms, the second meiotic division then proceeds rapidly to completion; in others, it halts at an intermediate stage and is not completed until after fertilization. In vertebrate eggs, for example, the second meiotic division is generally arrested at metaphase II until fertilization takes place. Metaphase II arrest is triggered by *cytostatic factor (CSF),* a biochemical activity present in the cytoplasm of mature eggs. CSF works by inhibiting the anaphase-promoting complex, whose activity is normally required for the transition from metaphase to anaphase (page 585). After an egg is fertilized, CSF undergoes inactivation. In the absence of CSF activity, the anaphase-promoting complex is free to trigger the transition from metaphase to anaphase, thereby allowing meiosis to be completed.

By the time meiosis is completed, the egg cell is fully mature and may even have been fertilized. The mature egg is a highly differentiated cell that is specialized for the task of producing a new organism in much the same sense that a muscle cell is specialized to contract or a red blood cell is specialized to transport oxygen. This inherent specialization of the egg is vividly demonstrated by the observation that even in the absence of fertilization by a sperm cell, many kinds of animal eggs can be stimulated to develop into a complete embryo by artificial treatments as simple as a pinprick. Hence, everything needed for programming the early stages of development must already be present in the egg.

Normally, of course, this developmental sequence is activated by interactions between sperm and egg, which trigger important biochemical changes in the egg. For example, in addition to the rapid formation of a physical barrier to the entry of additional sperm by the fertilized egg (page 405), fertilization simultaneously initiates a burst of metabolic activity in preparation for embryonic development. Although not described here, fertilization in plants involves similarly complex cellular and biochemical events.

Meiosis Generates Genetic Diversity

As we have pointed out, one of the main functions of meiosis is to preserve the chromosome number in sexually reproducing organisms. If it were not for meiosis, gametes would have as many chromosomes as other cells in the body, and the chromosome number would double each time gametes fused to form a new organism.

Equally important, however, is the role meiosis plays in generating genetic diversity in sexually reproducing populations. Meiosis is the point in the flow of genetic information where various combinations of chromosomes (and the alleles they carry) are assembled in gametes for potential passage to offspring. Although every gamete is haploid, and hence possesses one member of each pair of homologous chromosomes, the particular combination of paternal and maternal chromosomes in any given gamete is random. This randomness is generated by the random orientation of bivalents at metaphase I, where the paternal and maternal homologues of each pair can face either pole, independent of the orientations of the other bivalents. In human gametes, which contain 23 chromosomes, the end result is more than 8 million different combinations of maternal and paternal chromosomes!

Moreover, the crossing over that takes place between homologous chromosomes during prophase I generates additional genetic diversity among the gametes. By allowing the exchange of DNA segments between homologous chromosomes, crossing over generates more combinations of alleles than the random assortment of maternal and paternal chromosomes would create by itself. We will return to crossing over (recombination) later in the chapter. First, however, we turn to the historic experiments that revealed the genetic consequences of chromosome segregation and random assortment during meiosis. These experiments were carried out by Gregor Mendel before chromosomes were even known to exist.

Genetic Variability: Segregation and Assortment of Alleles

Most students of biology have heard of Gregor Mendel and the classic genetics experiments he conducted in a monastery garden. Mendel's findings, first published in 1865, laid the foundation for what we now know as *Mendelian genetics.* Working with the common garden pea, Mendel chose seven readily identifiable characters of pea plants and selected in each case two varieties of plants that displayed different forms of the character. For example, seed color was one character Mendel chose because he had one strain of peas with yellow seeds and another with green seeds (see Figure 20-1). He first established that each of the plant strains was **true-breeding** upon self-fertilization, which means that plants grown from his yellow seeds produced only yellow seeds and

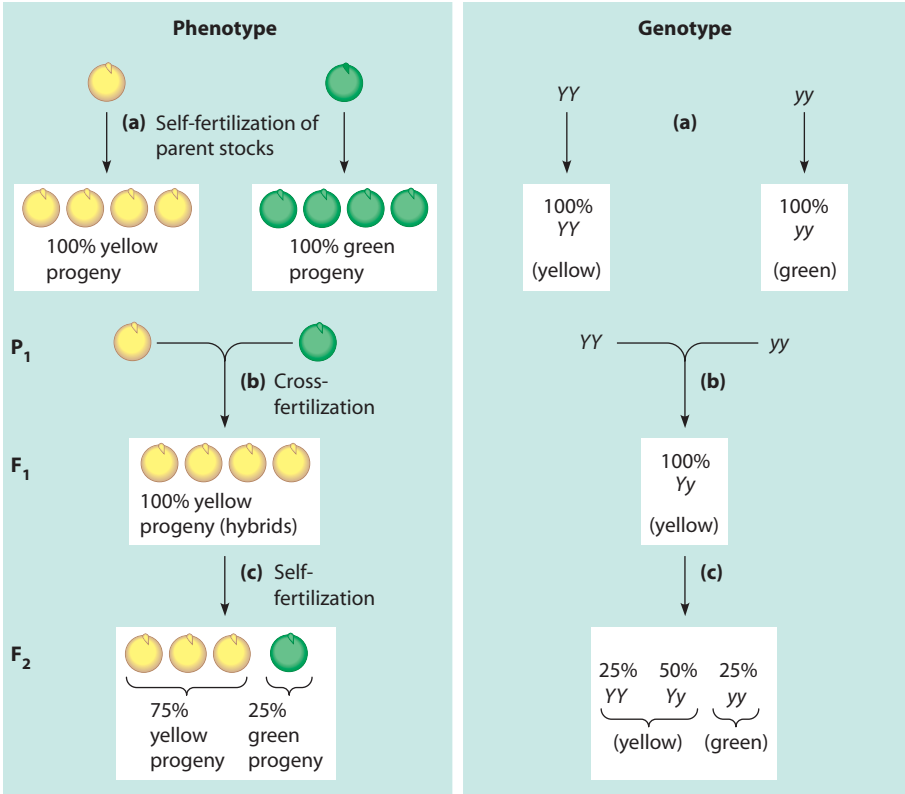


FIGURE 20-10 Genetic Analysis of Seed Color in Pea Plants. Genetic crosses were performed starting with two true-breeding strains of pea plants, one having yellow seeds and one having green seeds. The yellow-seed trait is dominant (allele *Y*); the green-seed trait is recessive (allele *y*). The resulting phenotypes of the progeny are shown on the left and their genotypes on the right. (The genotypes were deduced later.) **(a)** The parent stocks are homozygous for either the dominant *(YY)* or recessive *(yy)* trait and breed true upon self-fertilization. **(b)** When crossed, the parent stocks yield F_1 plants (hybrids) that are all heterozygous *(Yy)* and therefore show the dominant trait. **(c)** Upon self-fertilization, the F_1 plants produce yellow and green seeds in the F_2 generation in a ratio of 3:1. See Figures 20-11 and 20-12 for further analyses.

plants grown from his green seeds produced only green seeds (**Figure 20-10a**). Once this had been established, Mendel was ready to investigate the principles that govern the inheritance of such traits.

Information Specifying Recessive Traits Can Be Present Without Being Displayed

In his first set of experiments, Mendel *cross*-fertilized the true-breeding parental plants (the **P_1 generation**) to produce **hybrid** strains. The outcome of this experiment must have seemed mystifying at first: In every case, the resulting offspring—called the **F_1 generation**—exhibited one or the other of the parental traits, but never both. In other words, one parental trait was always dominant and the other was always recessive. In the case of seed color, for example, all the F_1 plants had yellow seeds (Figure 20-10b), indicating that yellow seed color is dominant.

During the next summer, Mendel allowed all the F_1 hybrids to self-fertilize. For each of the seven characters under study, he made the same surprising observation: The recessive trait that had seemingly disappeared in the F_1 generation reappeared among the progeny in the next generation, the **F_2 generation.** Moreover, for each of the seven characters, the ratio of dominant to recessive phenotypes in the progeny was always about 3:1. In the case of seed color, for example, plants grown from the yellow F_1 seeds produced about 75% yellow seeds and 25% green (Figure 20-10c).

This outcome was quite different from the behavior of the true-breeding yellow seeds of the parent strain, which had produced only yellow seeds when self-fertilized. Clearly, there was an important difference between the yellow seeds of the P_1 stock and the yellow seeds of the F_1 generation. They looked alike, but the former bred true whereas the latter did not.

Next, Mendel investigated the F_2 plants through self-fertilization (**Figure 20-11**). The F_2 plants exhibiting the recessive trait (green, in the case of seed color) always bred true (Figure 20-11c), suggesting they were genetically identical to the green-seeded P_1 strain that Mendel had begun with. F_2 plants with the dominant trait yielded a more complex pattern. One-third bred true for the dominant trait (Figure 20-11a) and therefore seemed identical to the P_1 plants with the dominant (yellow-seed) trait. The other two-thirds of the yellow-seeded F_2 plants produced progeny with both dominant and recessive phenotypes, in a 3:1 ratio (Figure 20-11b)—the same ratio that had arisen from the F_1 self-fertilization.

These results led Mendel to conclude that genetic information specifying the recessive trait must be present in the F_1 hybrid plants and seeds, even though the trait is not displayed. This conclusion was consistent with results from another set of experiments, in which F_1 hybrids were crossed with the original parent strains, a technique called **backcrossing** (**Figure 20-12**). Backcrossing F_1 hybrids to the dominant parent strain always produced progeny exhibiting the dominant trait (Figure 20-12a), whereas

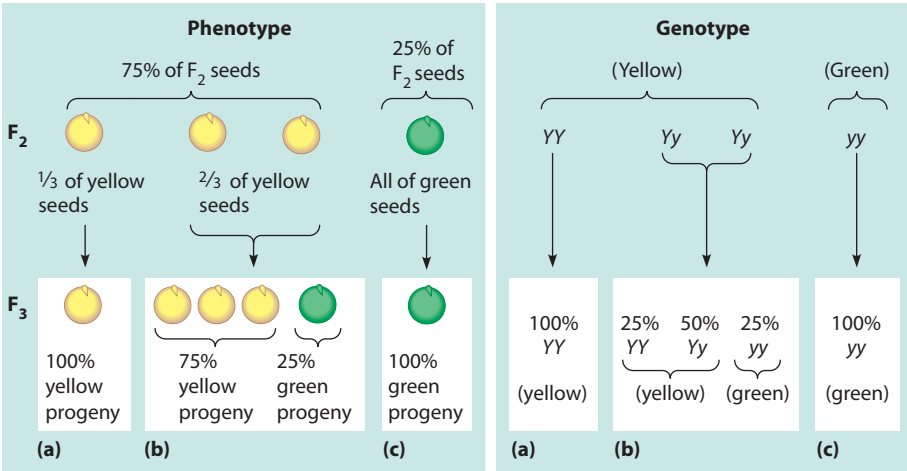


FIGURE 20-11 Analysis of F_2 Pea Plants by Self-Fertilization. The F_2 plants of Figure 20-10 were analyzed through self-fertilization. The phenotypic results are shown on the left and the genotypes (deduced later) on the right. **(a)** One-third of the yellow F_2 progeny of Figure 20-10 (25% of the total F_2 progeny) breed true for yellow seed color upon self-fertilization because they are genotypically *YY*. **(b)** Two-thirds of the yellow F_2 progeny (50% of the total F_2 progeny) yield yellow and green seeds upon self-fertilization, in a ratio of 3:1 (just as the F_2 plants of Figure 20-10 did upon self-fertilization). **(c)** All the green F_2 seeds (25% of the total F_2 progeny) breed true for green seed color upon self-fertilization because they are genotypically *yy*.

backcrossing to the recessive parent yielded a mixture of plants exhibiting dominant and recessive traits in a ratio of 1:1 (Figure 20-12b). Moreover, the dominant progeny from the latter cross behaved just like the F_1 hybrids: Upon self-fertilization, they gave rise to a 3:1 mixture of phenotypes (Figure 20-12c), and upon backcrossing to the recessive parent, they yielded a 1:1 ratio of dominant to recessive progeny (Figure 20-12d). (An alternative way of diagramming crosses, called the *Punnett square,* is shown in Problem 20-7 at the end of the chapter.)

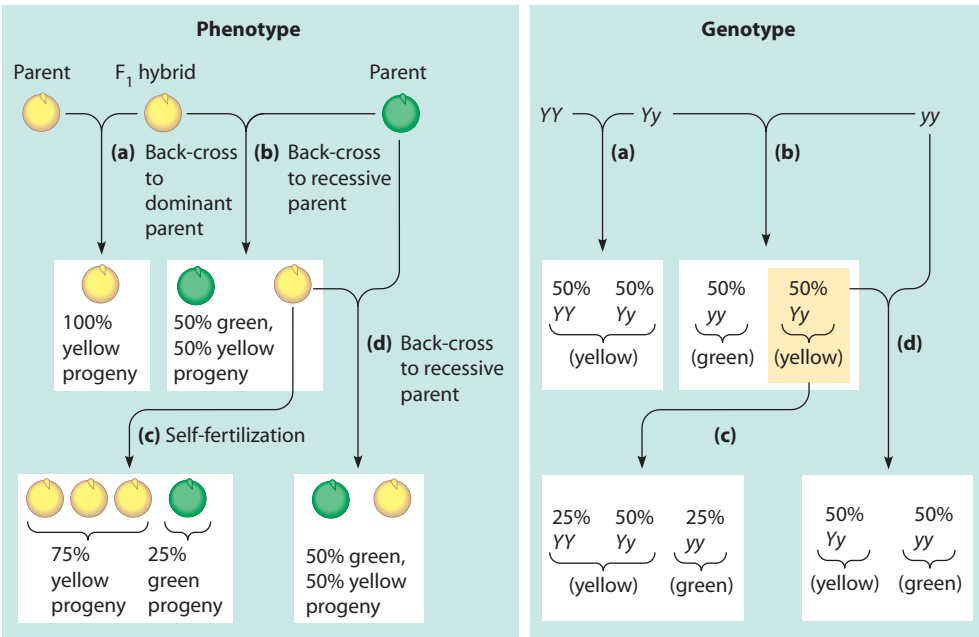


FIGURE 20-12 Analysis of F_1 Hybrids by Backcrossing. The F_1 hybrids of Figure 20-10 were analyzed by backcrossing to the parent (P_1) strains. The phenotypic results are shown on the left and the genotypes (deduced later) on the right. **(a)** Upon backcrossing of the F_1 hybrid *(Yy)* to the dominant parent *(YY)*, all the progeny have yellow seeds because the genotype is either *YY* or *Yy*. **(b)** Backcrossing to the recessive parent *(yy)* yields yellow *(Yy)* or green *(yy)* seeds in equal proportions. **(c)** These yellow-seeded progeny will give rise, upon self-fertilization, to a 3:1 mixture of yellow and green seeds (just as with the F_1 hybrids in Figure 20-10). **(d)** Backcrossing of the yellow-seeded progeny to the homozygous recessive parent again yields a 1:1 mixture of yellow and green seeds, as in the backcross of part b.

The Law of Segregation States That the Alleles of Each Gene Separate from Each Other During Gamete Formation

After a decade of careful work documenting the preceding patterns of inheritance, Mendel formulated several principles—now known as **Mendel's laws of inheritance**—that explained the results he had observed. The first of these principles was that phenotypic traits are determined by discrete "factors" that are present in most organisms as pairs of "determinants." Today, we call these "factors" *genes* and "determinants" *alleles* (alternative forms of genes). Mendel's conclusion seems almost self-evident to us, but it was an important assertion in his day. At that time, most scientists favored a *blending theory of inheritance,* a theory that viewed traits such as yellow and green seed color rather like cans of paint that are poured together to yield intermediate results. Other investigators had described the nonblending nature of inheritance before Mendel but without the accompanying data and mathematical analysis that were Mendel's special contribution. Mendel's breakthrough is especially impressive when we recall that he formulated his theory before anyone had seen chromosomes.

Of special importance to the development of genetics was Mendel's conclusion regarding the way genes are parceled out during gamete formation. According to his **law of segregation,** *the two alleles of a gene are distinct entities that segregate, or separate from each other, during the formation of gametes.* In other words, the two alleles retain their identities even when both are present in a hybrid organism, and they are then parceled out into separate gametes so they can emerge unchanged in later generations.

The Law of Independent Assortment States That the Alleles of Each Gene Separate Independently of the Alleles of Other Genes

In addition to the crosses already described, each focusing on a single pair of alleles, Mendel studied *multifactor* crosses between plants that differed in *several* characters. Besides differing in seed color, for example, the plants he crossed might differ in seed shape and flower position. As in his single-factor crosses, he used parent plants that were true-breeding (homozygous) for the characters he was testing and generated F_1 hybrids heterozygous for each character. He then self-fertilized these hybrids and determined how frequently the dominant and recessive forms of the various characters appeared among the progeny.

Mendel found that all possible combinations of traits appeared in the F_2 progeny, and he concluded that all possible combinations of the different alleles must therefore have been present among the F_1 gametes. Furthermore, based on the proportions of the various phenotypes detected in the F_2 generation, Mendel deduced that all possible combinations of alleles occurred in the gametes with equal frequency. In other words, *the two alleles of each gene segregate independently of the alleles of other genes.* This is the **law of independent assortment,** another cornerstone of genetics. Later, it would be shown that this law applies only to genes on different chromosomes or very far apart on the same chromosome.

Early Microscopic Evidence Suggested That Chromosomes Might Carry Genetic Information

Mendel's findings lay dormant in the scientific literature until 1900, when his paper was rediscovered almost simultaneously by three other European biologists. In the meantime, much had been learned about the cellular basis of inheritance. By 1875, for example, microscopists had identified chromosomes with the help of stains produced by the developing aniline dye industry. At about the same time, fertilization was shown to involve the fusion of sperm and egg nuclei, suggesting that the nucleus carries genetic information.

The first proposal that chromosomes might be the bearers of this genetic information was made in 1883. Within ten years, chromosomes had been studied in dividing cells and had been seen to split longitudinally into two apparently identical daughter chromosomes. This led to the realization that the number of chromosomes per cell remains constant during the development of an organism. With the invention of better optical systems, more detailed analysis of chromosomes became possible. Mitotic cell division was shown to involve the movement of identical daughter chromosomes to opposite poles, thereby ensuring that daughter cells would have exactly the same complement of chromosomes as their parent cell.

Against this backdrop came the rediscovery of Mendel's paper, followed almost immediately by three crucial studies that established chromosomes as the carriers of Mendel's factors. The investigators were Edward Montgomery, Theodor Boveri, and Walter Sutton. Montgomery's contribution was to recognize the existence of homologous chromosomes. From careful observations of insect chromosomes, he concluded that the chromosomes of most cells could be grouped into pairs, with one member of the pair of maternal origin and the other of paternal origin. He also noted that the two chromosomes of each type come together during synapsis in the "reduction division" (now called meiosis I) of gamete formation, a process that had been reported a decade or so earlier.

Boveri then added the crucial observation that each chromosome plays a unique genetic role. This idea came from studies in which sea urchin eggs were fertilized in the presence of a large excess of sperm, causing some eggs to be fertilized by two sperm. The presence of two sperm nuclei in a single egg leads to the formation of an abnormal mitotic spindle that does not distribute the chromosomes equally to the newly forming embryonic cells. The resulting embryos exhibit various types of developmental defects, depending on which particular chromosomes they

are missing. Boveri therefore concluded that each chromosome plays a unique role in development.

Sutton, meanwhile, was studying meiosis in the grasshopper. In 1902, he made the important observation that the orientation of each pair of homologous chromosomes (bivalent) at the spindle equator during metaphase I is purely a matter of chance. Any homologous pair, in other words, may lie with the maternal or paternal chromosome toward either pole, regardless of the positions of other pairs. Many different combinations of maternal and paternal chromosomes are therefore possible in the gametes produced by any given individual (note the similarity to Mendel's observation that all possible combinations of different alleles are present among the F_1 generation of gametes).

Chromosome Behavior Explains the Laws of Segregation and Independent Assortment

During 1902–1903, Sutton put the preceding observations together into a coherent theory describing the role of chromosomes in inheritance. This *chromosomal theory of inheritance* can be summarized as five main points:

1. Nuclei of all cells except those of the *germ line* (sperm and eggs) contain two sets of homologous chromosomes, one set of maternal origin and the other of paternal origin.
2. Chromosomes retain their individuality and are genetically continuous throughout an organism's life cycle.
3. The two sets of homologous chromosomes in a diploid cell are functionally equivalent, each carrying a similar set of genes.
4. Maternal and paternal homologues synapse during meiosis and then move to opposite poles of the division spindle, thereby becoming segregated into different cells.
5. The maternal and paternal members of different homologous pairs segregate independently during meiosis.

The chromosomal theory of inheritance provided a physical basis for understanding how Mendel's genetic factors could be carried, transmitted, and segregated. For example, the presence of two sets of homologous chromosomes in each cell parallels Mendel's suggestion of two determinants for each phenotypic trait. Likewise, the segregation of homologous chromosomes during the meiotic divisions of gamete formation provides an explanation for Mendel's law of segregation, and the random orientation of homologous pairs at metaphase I accounts for his law of independent assortment.

Figure 20-13 and **20-14** illustrate the chromosomal basis of Mendel's laws, using examples from his pea plants. The basis for the law of segregation is shown in Figure 20-13 for the alleles governing seed color in a heterozygous pea plant with genotype *Yy*. (Peas have seven pairs of chromosomes, but only the pair bearing the alleles for seed color is shown.) During meiosis the two homologous chromosomes, each with two sister chromatids, synapse during prophase I, align together at the spindle equator at metaphase I, and then segregate into separate daughter cells. The second meiotic division then separates sister chromatids so that each haploid cell ends up with only one allele for seed color, either *Y* or *y*.

The basis for the law of independent assortment is illustrated in Figure 20-14 for the chromosomes carrying the genes for seed color (alleles *Y* and *y*) and seed shape

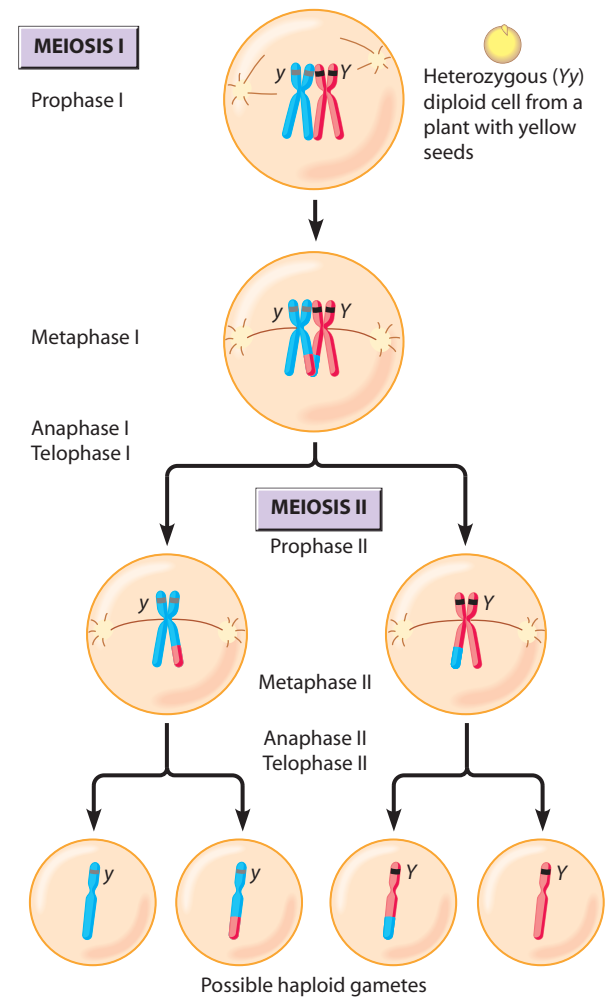


FIGURE 20-13 The Meiotic Basis for Mendel's Law of Segregation. Segregation of seed-color alleles during meiosis is illustrated for the case of a pea plant heterozygous for this character. Peas have seven pairs of chromosomes, but only the homologous pair bearing the seed-color alleles is shown here. During the first meiotic division (meiosis I), homologous chromosomes (each consisting of two sister chromatids) pair during prophase I, allowing crossing over to take place. The homologous chromosomes then align as a pair at the spindle equator during metaphase I and segregate into separate cells at anaphase I and telophase I. In meiosis II, sister chromatids segregate to different daughter cells. The result is four haploid daughter cells, each having one allele for seed color.

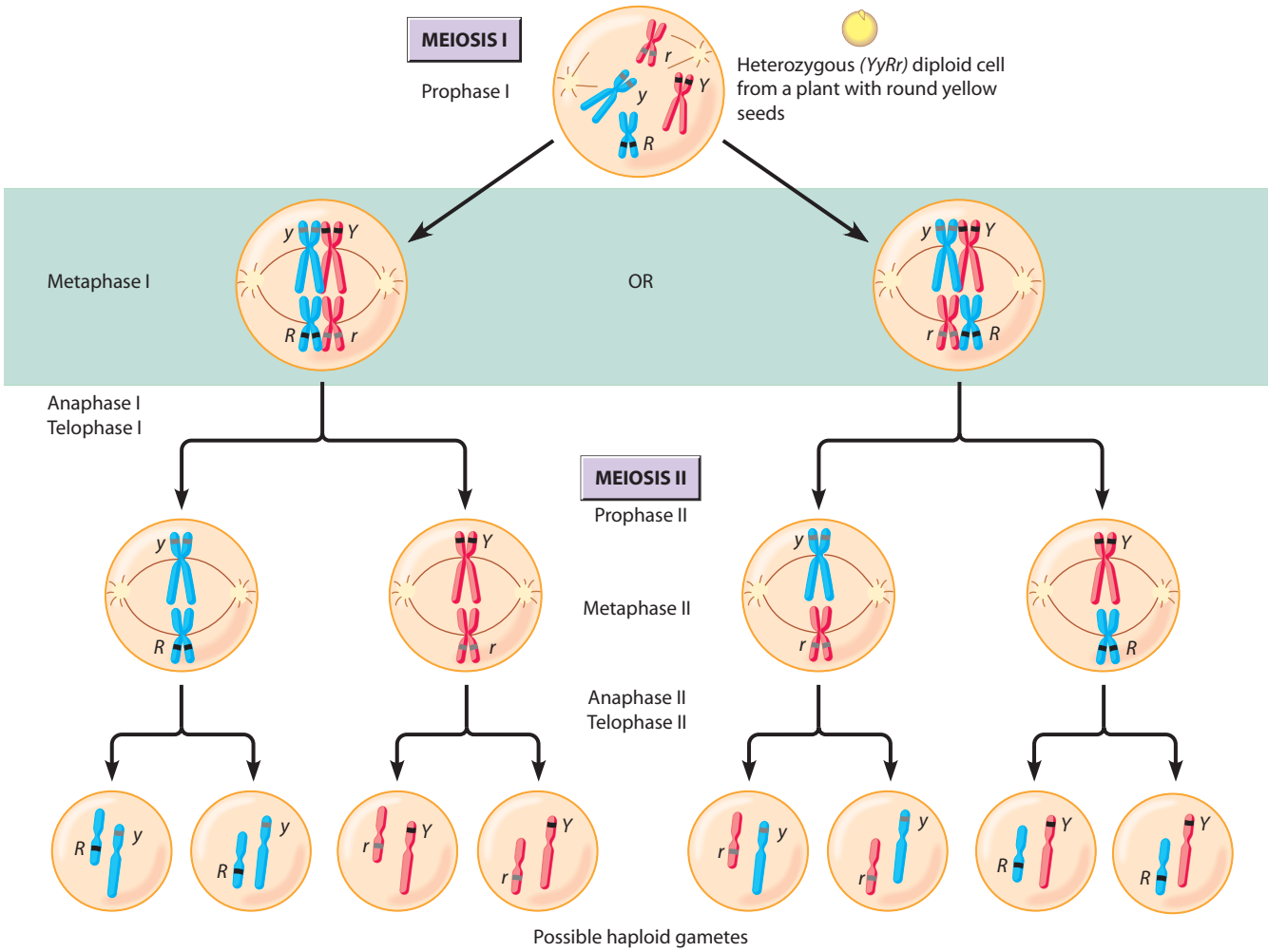


FIGURE 20-14 The Meiotic Basis for Mendel's Law of Independent Assortment. Independent assortment of the alleles of two genes on different chromosomes is illustrated by meiosis in a pea plant heterozygous for seed color *(Yy)* and seed shape *(Rr)*. Pea plants have seven pairs of chromosomes, but only the two pairs bearing the seed-color alleles and the seed-shape alleles are shown. During meiosis, segregation of the seed-color alleles occurs independently of segregation of the seed-shape alleles. The basis of this independent assortment is found at metaphase I, when the homologous pairs (bivalents) align at the spindle equator. Because each bivalent can face in either direction, there are two alternative situations: Either the paternal homologues (blue) can both face the same pole of the cell, with the maternal homologues (red) facing the other pole, or they can face different poles. The arrangement at metaphase I determines which homologues subsequently go to which daughter cells. Since the alternative arrangements occur with equal probability, all the possible combinations of the alleles therefore occur with equal probability in the gametes. For simplicity, chiasmata and crossing over are not shown in this diagram.

(alleles *R* and *r*, where *R* stands for round seeds, a dominant trait, and *r* for wrinkled seeds, a recessive trait). The explanation for independent assortment is that there are two possible, equally likely arrangements of the chromosome pairs at metaphase I. Of the *YyRr* cells that undergo meiosis, half will produce gametes like the four at the bottom left of the figure, and half will produce gametes like the four at the bottom right. Therefore, the *YyRr* plant will produce equal numbers of gametes of the eight types.

As we will discuss shortly, the law of independent assortment holds only for genes on *different* chromosomes (or for genes located far apart on the same chromosome). It is remarkable that Mendel happened to choose seven independently assorting characters in an organism that has only seven pairs of chromosomes. Mendel was also fortunate that each of the seven characters he chose to study turned out to be controlled by a single gene (pair of alleles). Perhaps he concentrated on pea strains that, in preliminary experiments, gave him the most consistent and comprehensible results.

The DNA Molecules of Homologous Chromosomes Have Similar Base Sequences

One of the five main points in the chromosomal theory of inheritance is the idea that homologous chromosomes are functionally equivalent, each carrying a similar set of genes. What does this mean in terms of our current understanding

of the molecular organization of chromosomes? Simply put, it means that homologous chromosomes have DNA molecules whose base sequences are almost, but not entirely, identical. Thus, homologous chromosomes typically carry the same genes in exactly the same order. However, minor differences in base sequence along the chromosomal DNA molecule can create different alleles of the same gene. Such differences arise by mutation, and the different alleles for a gene that we find in a population—whether of pea plants or people—arise from mutations that have gradually occurred in an ancestral gene. Alleles are usually "expressed" by transcription into RNA and translation into proteins, and it is the behavior of these proteins that ultimately creates an organism's phenotype. As we will see in Chapter 21, a change as small as a single DNA base pair can create an allele that codes for an altered protein that is different enough to cause an observable change in an organism's phenotype—in fact, such a DNA alteration can be lethal.

The underlying similarity in the base sequences of their DNA molecules—*DNA homology*—is thought to explain the ability of homologous chromosomes to undergo synapsis (close pairing) during meiosis and is essential for normal crossing over. One popular model for synapsis holds that the correct alignment of homologous chromosomes is brought about before completion of the synaptonemal complex by base-pairing interactions between matching regions of DNA in the two chromosomes. Support for this idea has come from studies showing that the chromosomes of some organisms possess special DNA sequences, called *pairing sites,* that promote synapsis between chromosomes when the same pairing site is present in two chromosomes. Protein components of the synaptonemal complex also play a role in facilitating the pairing between matching regions in the DNA of homologous chromosomes. After the synaptonemal complex is fully formed, DNA recombination is completed at these (and perhaps other) sites.

Genetic Variability: Recombination and Crossing Over

Segregation and independent assortment of homologous chromosomes during the first meiotic division lead to random assortment of the alleles carried by different chromosomes, as was shown in Figure 20-14 for seed shape and color in pea plants. **Figure 20-15a** summarizes the outcome of meiosis for a generalized version of the same situation. Here we have a diploid organism heterozygous for two genes on nonhomologous chromosomes, with the allele pairs called *Aa* and *Bb.* Meiosis in such an organism will produce gametes in which allele *A* is just as likely to occur with allele *B* as it is with allele *b,* and *B* is just as likely to occur with *A* as it is with *a.* But what happens if two genes, *D* and *E,* reside on the same chromosome? In that case, alleles *D* and *E* will routinely be linked together in the same gamete, as will alleles *d* and *e* (Figure 20-15b).

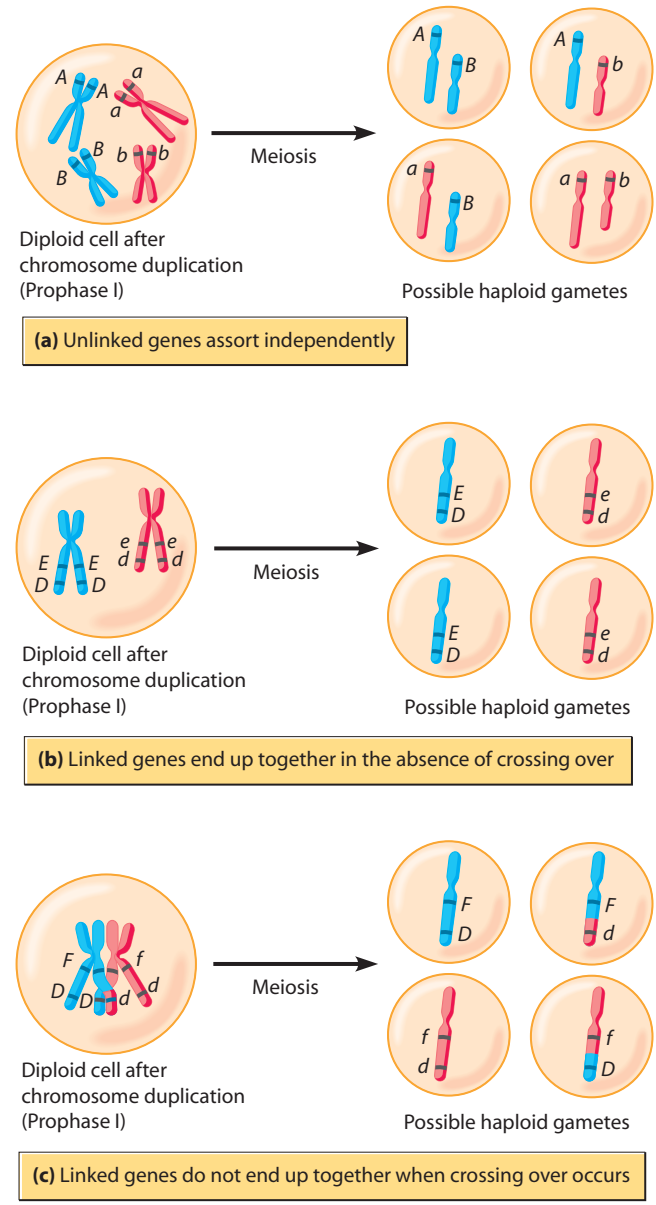


FIGURE 20-15 Segregation and Assortment of Linked and Unlinked Genes. In this figure, *A* and *a* are alleles of one gene, *B* and *b* are alleles of another gene, and so forth. **(a)** Alleles of genes on different chromosomes segregate and assort independently during meiosis; allele *A* is as likely to occur in a gamete with allele *B* as it is with allele *b.* **(b)** Alleles of genes on the same chromosome remain linked during meiosis in the absence of crossing over; in this case, allele *D* will occur routinely in the same gamete with allele *E* but not with allele *e.* **(c)** Alleles of genes on the same chromosome can become interchanged when crossing over takes place, so that allele *D* occurs not only with allele *F* but also with allele *f* and so forth.

But even for genes on the same chromosome, some scrambling of alleles can take place because of the phenomenon of crossing over, which leads to genetic recombination. Genetic recombination involves the exchange of genetic material between homologous chromosomes during prophase I of meiosis when the homologues are synapsed, creating chromosomes exhibiting new combina-

tions of alleles (Figure 20-15c). Recombination was originally discovered in studies with the fruit fly *Drosophila melanogaster,* conducted by Thomas Hunt Morgan and his colleagues beginning around 1910. We will therefore turn to Morgan's work to investigate recombination, beginning with his discovery of linkage groups.

Chromosomes Contain Groups of Linked Genes That Are Usually Inherited Together

The fruit flies used by Morgan and his colleagues had certain advantages over Mendel's pea plants as objects of genetic study, not the least of which was the fly's relatively brief generation time (about two weeks versus several months for pea plants). Unlike Mendel's peas, however, the fruit flies did not come with a ready-made variety of phenotypes and genotypes. Whereas Mendel was able to purchase seed stocks of different true-breeding varieties, the only type of fruit fly initially available to Morgan was what has come to be known as the **wild type,** or "normal" organism. Morgan and his colleagues therefore had to generate variants—that is, mutants—for their genetic experiments.

Morgan and his coworkers began by breeding large numbers of flies and then selecting mutant individuals having phenotypic modifications that were heritable. Later, X-irradiation was used to enhance the mutation rate, but in their early work Morgan's group depended entirely on spontaneous mutations. Within five years, they were able to identify about 85 different mutants, each carrying a mutation in a different gene. Each of these mutants could be propagated as a laboratory stock and used for matings as needed.

One of the first discoveries made by Morgan and his colleagues as they began analyzing their mutants was that, unlike the genes Mendel had studied in peas, the mutant fruit fly genes did not all assort independently. Instead, some genes behaved as if they were linked together, and for such genes, the new combinations of alleles predicted by Mendel were infrequent or even nonexistent. In fact, it was soon recognized that fruit fly genes can be classified into four **linkage groups,** each group consisting of a collection of **linked genes** that are usually inherited together. Morgan quickly realized that the number of linkage groups was the same as the number of different chromosomes in the organism (the haploid chromosome number for *Drosophila* is four). The conclusion he drew was profound: Each chromosome is the physical basis for a specific linkage group. Mendel had not observed linkage, because the genes he studied either resided on different chromosomes or were far apart on the same chromosome and therefore behaved as if they were not linked to each other.

Homologous Chromosomes Exchange Segments During Crossing Over

Although the genes they discovered could all be organized into linkage groups, Morgan and his colleagues found that linkage within such groups was incomplete. Most of the time, genes known to be linked (and therefore on the same chromosome) assorted together, as would be expected. Sometimes, however, two or more such traits would appear in the offspring in nonparental combinations. This phenomenon of less-than-complete linkage was called *recombination* because the different alleles appeared in new and unexpected associations in the offspring.

To explain such recombinant offspring, Morgan proposed that homologous chromosomes can exchange segments, presumably by some sort of breakage-and-fusion event, as illustrated in **Figure 20-16a**. By this process, which Morgan termed *crossing over,* a particular

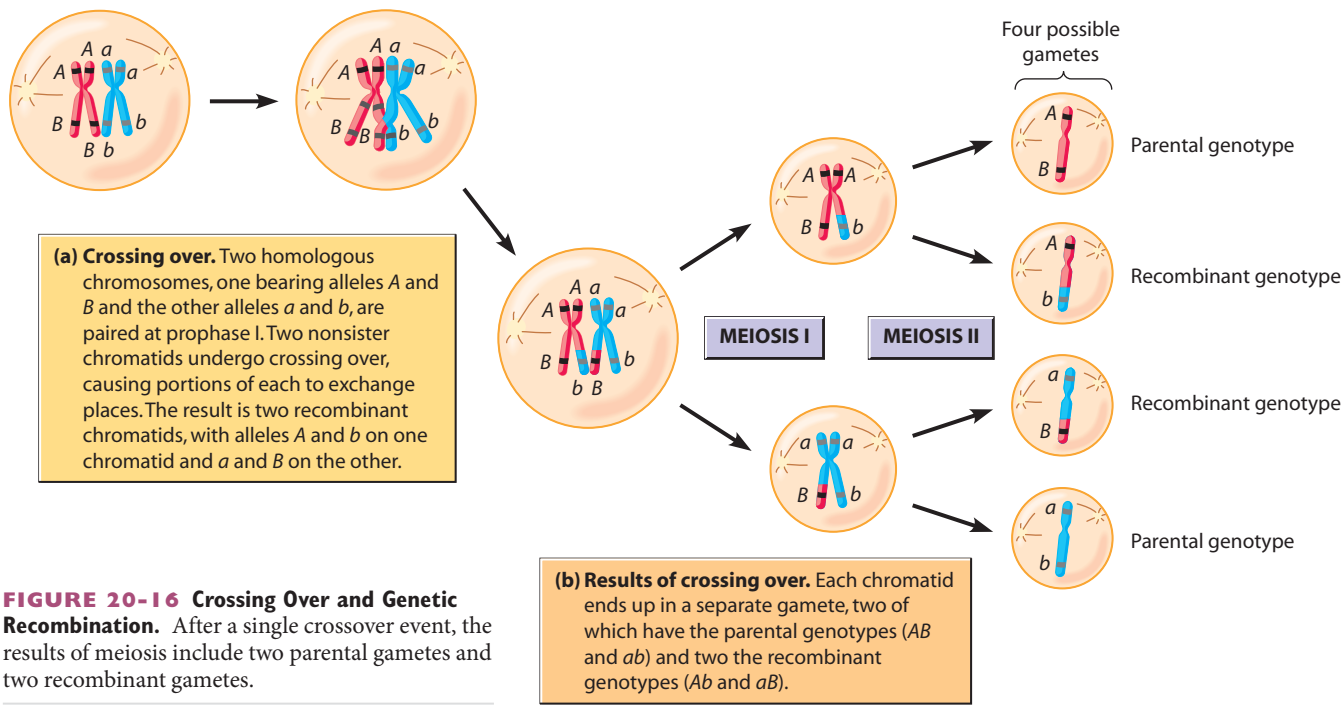


FIGURE 20-16 Crossing Over and Genetic Recombination. After a single crossover event, the results of meiosis include two parental gametes and two recombinant gametes.

allele or group of alleles initially present on one member of a homologous pair of chromosomes could be transferred to the other chromosome in a reciprocal manner.

In the example of Figure 20-16a, two homologous chromosomes, one with alleles *A* and *B* and the other with alleles *a* and *b,* lie side by side at synapsis. Portions of an *AB* chromatid and a nonsister *ab* chromatid then exchange DNA segments, thereby producing two recombinant chromatids—one with alleles *A* and *b* and the other with alleles *a* and *B.* Each of the four chromatids ends up in a different gamete at the end of the second meiotic division, so the products of meiosis will include two *parental* gametes and two *recombinant* gametes, assuming a single crossover event (Figure 20-16b).

We now know that crossing over takes place during the pachytene stage of meiotic prophase I (see Figure 20-6), when sister chromatids are packed tightly together and it is difficult to observe what is happening. As the chromatids begin to separate at diplotene, each of the four chromatids in a bivalent can be identified as belonging to one or the other of the two homologues. Wherever crossing over has taken place between nonsister chromatids, the two homologues remain attached to each other, forming a chiasma.

At the first meiotic metaphase, homologous chromosomes are almost always held together by at least one chiasma; if not, they may not segregate properly. Many bivalents contain multiple chiasmata. Human bivalents, for example, typically have two or three chiasmata because multiple crossover events routinely occur between paired homologues. To be genetically significant, crossing over must involve nonsister chromatids. In some species, exchanges between sister chromatids are also observed, but such exchanges have no genetic consequences because sister chromatids are genetically identical.

Gene Locations Can Be Mapped by Measuring Recombination Frequencies

Eventually, it became clear to Morgan and others that the frequency of recombinant progeny differed for different pairs of genes within each linkage group. This observation suggested that the frequency of recombination between two genes might be a measure of how far the genes are located from each other since genes located near each other on a given chromosome would be less likely to become separated by a crossover event than would genes that are far apart. This insight led Alfred Sturtevant, an undergraduate student in Morgan's lab, to suggest in 1911 that recombination data could be used to determine where genes are located along the chromosomes of *Drosophila.* In other words, a chromosome had come to be viewed as a linear string of genes whose positions can be determined on the basis of recombination frequencies.

Determining the sequential order and spacing of genes on a chromosome based on recombinant frequencies is called **genetic mapping.** In the construction of such maps, the recombinant frequency is the map distance expressed in *map units (centimorgans).* If, for example, the alleles in Figure 20-16b appear among the progeny in their parental combination (*AB* and *ab*) 85% of the time and in the recombinant combinations (*Ab* and *aB*) 15% of the time, we conclude that the two genes are linked (are on the same chromosome) and are 15 map units apart. This approach has been used to map the chromosomes of many species of plants and animals, as well as bacteria and viruses. However, because bacteria and viruses do not reproduce sexually, the methods used to generate recombinants are somewhat different.

Genetic Recombination in Bacteria and Viruses

Because it requires crossing over between homologous chromosomes, you might expect genetic recombination to be restricted to sexually reproducing organisms. Sexual reproduction provides an opportunity for crossing over once every generation when homologous chromosomes become closely juxtaposed during the meiotic divisions that produce gametes. In contrast, bacteria and viruses have haploid genomes and reproduce asexually, with no obvious mechanism for regularly bringing together genomes from two different parents.

Nonetheless, viruses and bacteria are still capable of genetic recombination. In fact, recombination data permitted the extensive mapping of viral and bacterial genomes well before the advent of modern DNA technology. To understand how recombination takes place despite a haploid genome, we need to examine the mechanisms that allow two haploid genomes, or portions of genomes, to be brought together within the same cell.

Co-infection of Bacterial Cells with Related Bacteriophages Can Lead to Genetic Recombination

Much of our early understanding of genes and recombination at the molecular level, as well as the vocabulary we use to express that information, came from experiments involving bacteriophages, particularly T-even phages and phage λ (see Box 18A, page 508). Although phages are haploid and do not reproduce sexually, genetic recombination between related phages can take place when individual bacterial cells are simultaneously infected by different versions of the same phage. **Figure 20-17** illustrates an experiment in which bacterial cells are co-infected by two related types of T4 phage. As the phages replicate in the bacterial cell, their DNA molecules occasionally become juxtaposed in ways that allow DNA segments to be exchanged between homologous regions. The resulting recombinant phage arises at a frequency that depends on the distance between the genes under study, just as in diploid organisms: The farther apart the genes, the greater the likelihood of recombination between them.

In phage recombination, crossing over involves relatively short, naked DNA molecules, rather than chromatids. The simplicity of this situation has facilitated research on the molecular mechanism of recombination and the proteins that catalyze the process. Phage recombination involves the precise alignment of homologous DNA molecules at the region of crossing over—a requirement that presumably holds for the recombination of eukaryotic and prokaryotic DNA as well.

Transformation and Transduction Involve Recombination with Free DNA or DNA Brought into Bacterial Cells by Bacteriophages

In bacteria, several mechanisms exist for recombining genetic information. One such mechanism has already been mentioned in Chapter 18, where we discussed the experiments with rough and smooth strains of pneumococcal bacteria that led Oswald Avery to conclude that bacterial cells can be transformed from one genetic type to another by exposing them to purified DNA. This ability of a bacterial cell to take up DNA molecules and to incorporate some of that DNA into its own genome is called **transformation** (**Figure 20-18a**). Although initially described as a laboratory technique for artificially introducing DNA into bacterial cells, transformation is now recognized as a natural mechanism by which some (though by no means all) kinds of bacteria acquire genetic information when they have access to DNA from other cells.

A second mechanism for genetic recombination in bacteria, called **transduction,** involves DNA that has been brought into a bacterial cell by a bacteriophage. Most phages contain only their own DNA, but occasionally a phage will incorporate some bacterial host cell DNA sequences into its progeny particles. Such a phage particle can then infect another bacterium, acting like a syringe carrying DNA from one bacterial cell to the next (Figure 20-18b). Phages capable of carrying host cell DNA from one cell to another are called *transducing phages.*

The transducing phage known as P1, which infects *E. coli,* has been especially useful for gene mapping. The amount of DNA that will fit into a phage particle is small compared with the size of the bacterial genome. Two bacterial genes—or more generally, genetic markers (specific DNA sequences)—must therefore be close together for both to be simultaneously carried into a bacterial cell by a single phage particle. This is the basis of *cotransductional mapping,* in which the proximity of one marker to another is determined by measuring how frequently the markers accompany each other in a transducing phage particle. The closer two markers are, the more likely they are to be cotransduced into a bacterial cell. Studies using the transducing phage P1 have revealed that markers cannot be cotransduced if they are separated in the bacterial DNA by more than about 10^5 base pairs. This finding agrees with the observation that the P1 phage has a genome of about that size.

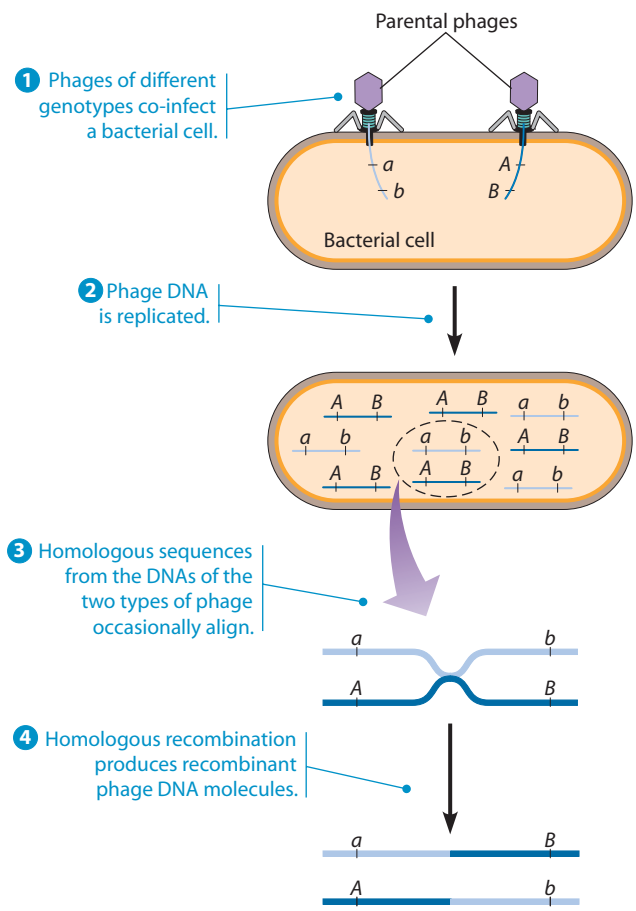


FIGURE 20-17 Genetic Recombination of Bacteriophages. Two phage populations with different genotypes co-infect a bacterial cell, thereby ensuring the simultaneous presence of both phage genomes in the same cell. One phage carries the mutant alleles *a* and *b,* while the other has alleles *A* and *B.* As the phage DNAs replicate, they occasionally become aligned and crossing over takes place, generating recombinant phage DNA molecules exhibiting *aB* and *Ab* genotypes. The frequency of occurrence of these recombinant genotypes provides a measure of the distance between genes.

Conjugation Is a Modified Sexual Activity That Facilitates Genetic Recombination in Bacteria

In addition to transformation and transduction, some bacteria also transfer DNA from one cell to another by **conjugation.** As the name suggests, conjugation resembles a mating in that one bacterium is clearly identifiable as the donor (often called a "male") and another as the recipient ("female"). Although conjugation resembles a sexual process, this mode of DNA transfer is not an inherent part of the bacterial life cycle, and it usually involves only a portion of the genome; therefore conjugation does not qualify as true sexual reproduction. The existence of conjugation was postulated in 1946 by Joshua Lederberg and Edward L. Tatum, who were the first to show that genetic recombination occurs in bacteria. They also established that physical contact between two cells is necessary for conjugation to take place. We now understand that conjugation involves

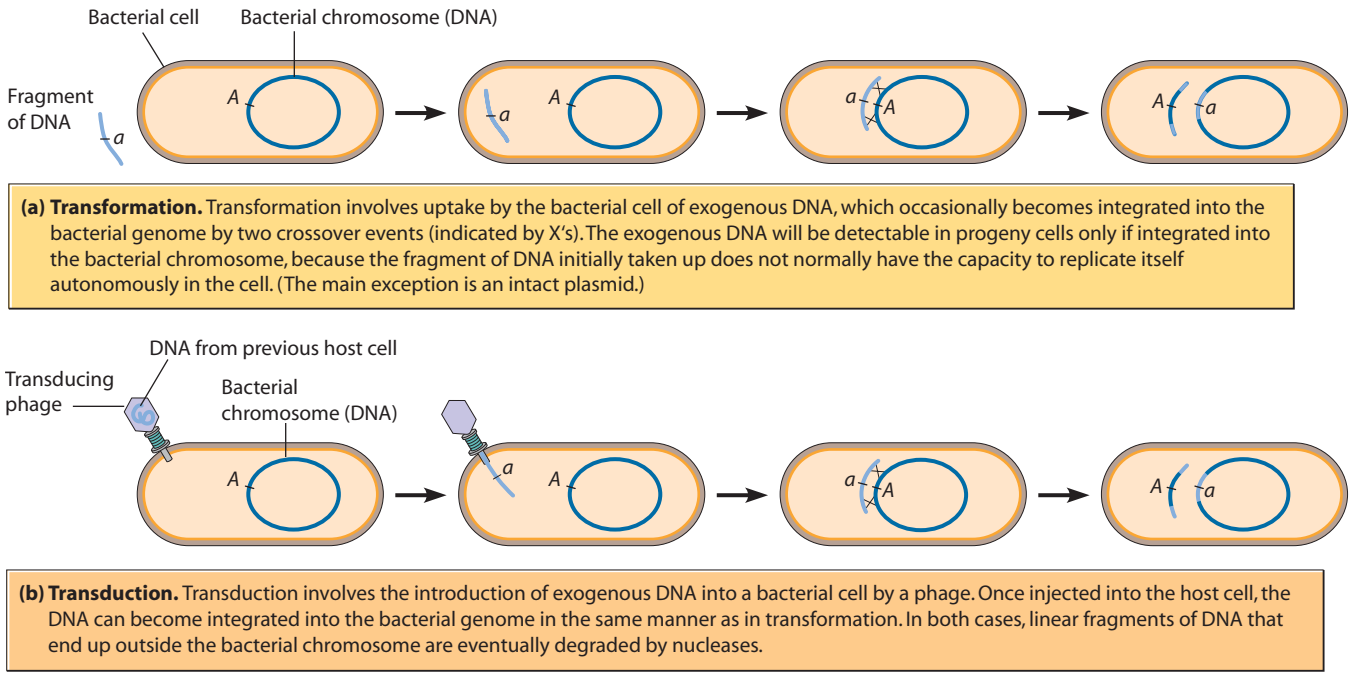


FIGURE 20-18 Transformation and Transduction in Bacterial Cells. Transformation and transduction are mechanisms by which bacteria incorporate external genetic information derived either from free DNA or from DNA brought into bacterial cells by bacteriophages. In this figure, the letters *A* and *a* represent alleles of the same gene.

the directional transfer of DNA from the donor bacterium to the recipient bacterium. Let's look at some details of how the process works.

The F Factor. The presence of a DNA sequence called the **F factor** (F for fertility) enables an *E. coli* cell to act as a donor during conjugation. The F factor can take the form of either an independent, replicating plasmid (page 530) or a segment of DNA within the bacterial chromosome. Donor bacteria containing the F factor in its plasmid form are designated F^+, whereas recipient cells, which usually lack the F factor completely, are designated F^-. Donor cells develop long, hairlike projections called **sex pili** (singular: **pilus**) that emerge from the cell surface (**Figure 20-19a**). The end of each sex pilus contains molecules that selectively bind to the surface of recipient cells, thereby leading to the formation of a transient cytoplasmic **mating bridge** through which DNA is transferred from donor cell to recipient cell (Figure 20-19b).

When a donor cell contains an F factor in its plasmid form, a copy of the plasmid is quickly transferred to the recipient cell during conjugation, converting the recipient cell from F^- to F^+ (**Figure 20-20a**). Transfer always begins at a point on the plasmid called its **origin of transfer,** represented in the figure by an arrowhead. During transfer of an F factor to an F^- cell, the donor cell does not lose its F^+ status because the F factor is replicated in close association with the transfer process, allowing a copy of the F plasmid to remain behind in the donor cell. As a result, mixing an F^+ population of bacteria with F^- cells will eventually lead to a population of cells that is entirely F^+. "Maleness" is in a sense infectious, and the F factor is responsible for this behavior.

Hfr Cells and Bacterial Chromosome Transfer. Thus far, we have seen that donor and recipient cells are defined by the presence or absence of the F factor, which is in turn transmitted by conjugation. But how do recombinant bacteria arise by this means? The answer is that the F factor—while usually present as a plasmid—can sometimes become integrated into the bacterial chromosome, as shown in Figure 20-20b. (Integration results from crossing over between short DNA sequences in the chromosome and similar sequences in the F factor.) Chromosomal integration of the F factor converts an F^+ donor cell into an **Hfr cell,** which is capable of producing a *h*igh *f*requency of *r*ecombination in further matings because it can now transfer *genomic DNA* during conjugation.

When an Hfr bacterium is mated to an F^- recipient, DNA is transferred into the recipient cell (Figure 20-20c). But instead of transferring just the F factor itself, the Hfr cell transfers at least part (and occasionally all) of its chromosomal DNA, retaining a copy, as in F^+ DNA transfer. Transfer begins at the origin of transfer within the integrated F factor and proceeds in a direction dictated by the orientation of the F factor within the chromosome. Note that the chromosomal DNA is transferred in a linear form, with a small part of the F factor at the leading end and the remainder at the trailing end. Because the F factor is split in this way, only recipient cells that receive a complete

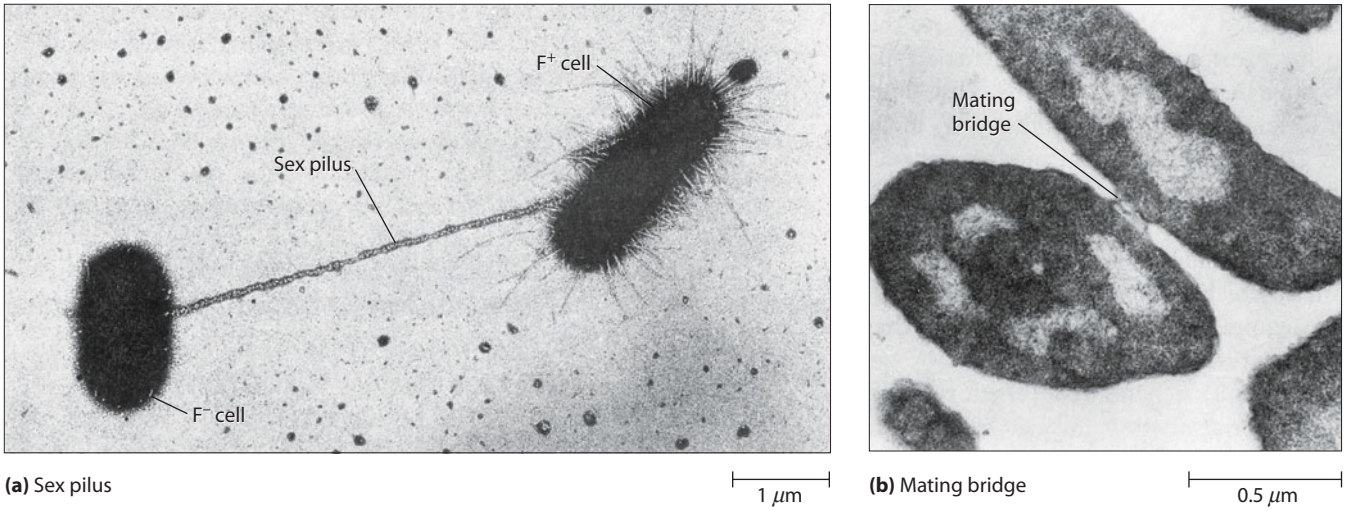
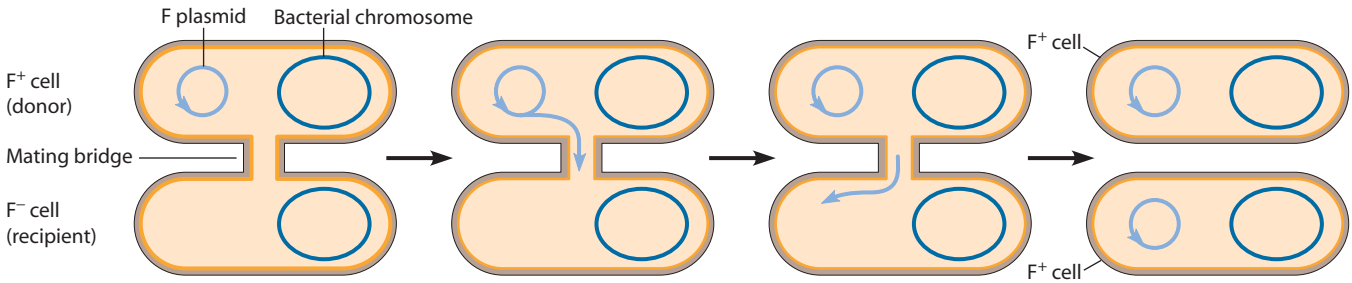
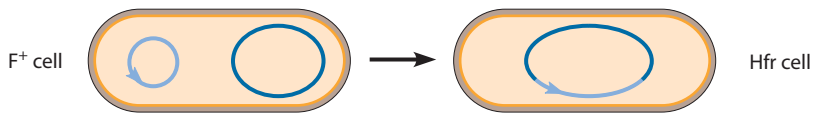


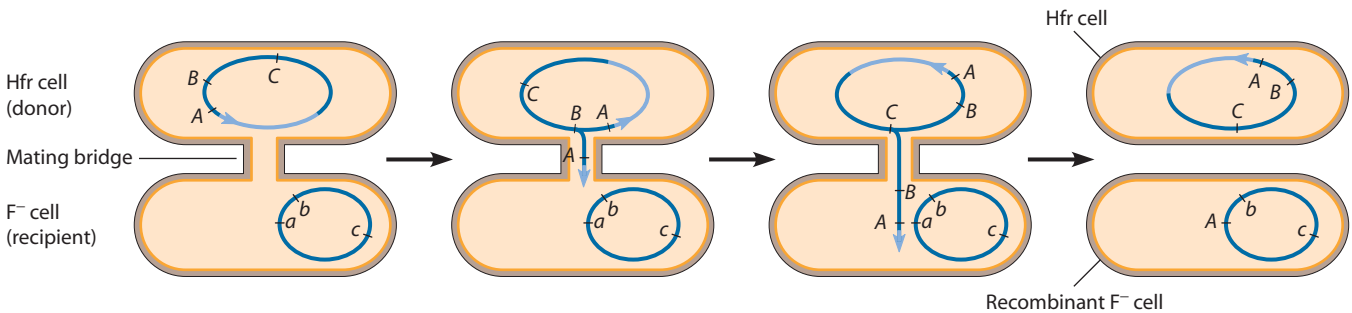
FIGURE 20-19 The Cellular Apparatus for Bacterial Conjugation. **(a)** The donor bacterial cell on the right, an F^+ cell, has many slender appendages, called pili, on its surface. Some of these pili are sex pili, including the very long pilus leading to the other cell, an F^- cell. Made of protein encoded by a gene on the F factor, sex pili enable a donor cell to attach to a recipient cell. **(b)** Subsequently, a cytoplasmic mating bridge forms, through which DNA is passed from the donor cell to the recipient cell (TEMs).



(a) Conjugation between F^+ and F^- cells. The transfer of a copy of the F factor plasmid from an F^+ donor bacterium to an F^- recipient converts the F^- cell into an F^+ cell. Plasmid transfer occurs through a mating bridge and begins at the F factor's origin of transfer, indicated by the arrowhead.



(b) Conversion of an F^+ cell into an Hfr cell. Integration of the F factor into the bacterial chromosome converts an F^+ cell into an Hfr cell.



(c) Conjugation between an Hfr cell and an F^- cell. Transferring a copy of the Hfr genome into an F^- cell begins with the origin of transfer on the integrated F factor. Cells rarely remain in contact long enough for the entire bacterial chromosome to be transferred. Once inside the F^- cell, parts of the Hfr DNA recombine with the DNA of the F^- cell. Uppercase letters represent alleles carried by the Hfr; lowercase letters represent corresponding alleles in the F^- cell. In the last step, allele *A* from the Hfr is recombined into the F^- cell's DNA in place of its *a* allele.

FIGURE 20-20 DNA Transfer by Bacterial Conjugation. **(a)** The presence of the F factor plasmid enables an *E. coli* cell to act as a plasmid donor during conjugation. **(b)** Chromosomal integration of the F factor creates an Hfr cell that can **(c)** transfer genomic DNA during conjugation.

bacterial chromosome from the Hfr donor actually become Hfr cells themselves. Transfer of the whole chromosome is extremely rare, however, because it takes about 90 minutes. Usually, mating contact is spontaneously disrupted before transfer is complete, leaving the recipient cell with only a portion of the Hfr chromosome, as shown in Figure 20-20c. As a result, genes located close to the origin of transfer on the Hfr chromosome are the most likely to be transmitted to the recipient cell.

Once a portion of the Hfr chromosome has been introduced into a recipient cell by conjugation, it can recombine with regions of the recipient cell's chromosomal DNA that are homologous (similar) in sequence. The recombinant bacterial chromosomes generated by this process contain some genetic information derived from the donor cell and some from the recipient. Only donor DNA sequences that are successfully integrated by this recombination mechanism survive in the recipient cell and its progeny. Donor DNA that is not integrated during recombination, as well as DNA removed from the recipient chromosome during the recombination process, is eventually degraded by nucleases.

The correlation between the position of a gene within the bacterial chromosome and its likelihood of transfer can be used to map genes with respect to the origin of transfer and therefore with respect to one another. For example, if gene *A* of an Hfr cell is transferred in conjugation 95% of the time, gene *B* 70% of the time, and gene *C* 55% of the time, then the sequence of genes is *A-B-C,* with gene *A* closest to the origin of transfer. Moreover, since the daughter cells of the recipient bacterium are recombinants, they can be used for genetic analysis. Typically, a cross is made between Hfr and F^- strains that differ in two or more genetic properties. After conjugation has taken place, the cells are plated on a nutrient medium on which recombinants can grow but "parent" strains cannot, thereby allowing the recombinants to be detected and recombinant frequencies to be calculated.

Molecular Mechanism of Homologous Recombination

We have now described five different situations in which genetic information can be exchanged between homologous DNA molecules: (1) prophase I of meiosis associated with gametogenesis in eukaryotes, (2) co-infection of bacteria with related bacteriophages, (3) transformation of bacteria with DNA, (4) transduction of bacteria by transducing phages, and (5) bacterial conjugation. Despite their obvious differences, all five situations share a fundamental feature: Each involves **homologous recombination** in which genetic information is exchanged between DNA molecules exhibiting extensive sequence similarity. We are now ready to discuss the molecular mechanisms underlying this type of recombination. Since the principles involved appear to be quite similar in prokaryotes and eukaryotes, we will use examples from both types of organisms.

DNA Breakage and Exchange Underlies Homologous Recombination

Shortly after it was first discovered that genetic information is exchanged between chromosomes during meiosis, two theories were proposed to explain how this might occur. The *breakage-and-exchange model* postulated that breaks occur in the DNA molecules of two adjoining chromosomes, followed by exchange and rejoining of the broken segments. In contrast, the *copy-choice model* proposed that genetic recombination occurs while DNA is being replicated. According to the latter view, DNA replication begins by copying a DNA molecule located in one chromosome and then switches at some point to copying the DNA located in the homologous chromosome. The net result would be a new DNA molecule containing information derived from both chromosomes. One of the more obvious predictions made by the copy-choice model is that DNA replication and genetic recombination should happen at the same time. When subsequent studies revealed that DNA replication takes place during S phase while recombination typically occurs during prophase I, the copy-choice idea had to be rejected as a general model of meiotic recombination.

The first experimental evidence providing support for the breakage-and-exchange model was obtained in 1961 by Matthew Meselson and Jean Weigle, who employed phages of the same genetic type labeled with either the heavy (^{15}N) or light (^{14}N) isotope of nitrogen. Simultaneous infection of bacterial cells with these two labeled strains of the same phage resulted in the production of recombinant phage particles containing genes derived from both phages. When the DNA from these recombinant phages was examined, it was found to contain a mixture of ^{15}N and ^{14}N (**Figure 20-21**). Since these experiments were performed under conditions that prevented any new DNA from being synthesized, the recombinant DNA molecules must have been produced by breaking and rejoining DNA molecules derived from the two original phages.

Subsequent experiments involving bacteria whose chromosomes had been labeled with either ^{15}N or ^{14}N revealed that DNA containing a mixture of both isotopes is also produced during genetic recombination between bacterial chromosomes. Moreover, when such recombinant DNA molecules are heated to dissociate them into single strands, a mixture of ^{15}N and ^{14}N is detected in each DNA strand; hence, the DNA double helix must be broken and rejoined during recombination.

A similar conclusion emerged from experiments performed shortly thereafter on eukaryotic cells by J. Herbert Taylor. In these studies, cells were briefly exposed to ^{3}H-thymidine during the S phase preceding the last mitosis prior to meiosis, producing chromatids containing one radioactive DNA strand per double helix. During the following S phase, DNA replication in the absence of ^{3}H-thymidine generated chromosomes containing one

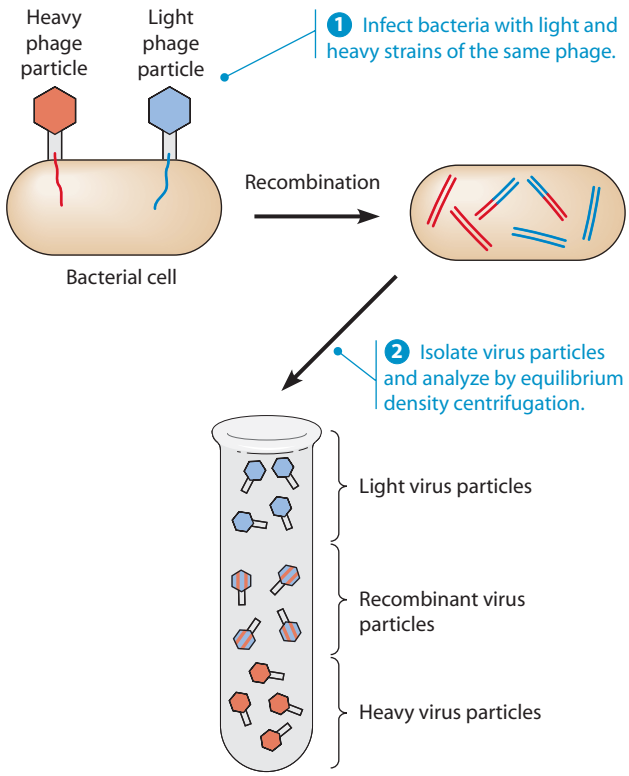


FIGURE 20-21 Evidence for DNA Breakage and Exchange During Bacteriophage Recombination. Bacterial cells were infected with two strains of the same phage, one labeled with ^{15}N and the other with ^{14}N. After recombination, the DNA from the recombinant phages was found to contain both ^{15}N and ^{14}N, supporting the idea that recombination involves the breaking and rejoining of DNA molecules.

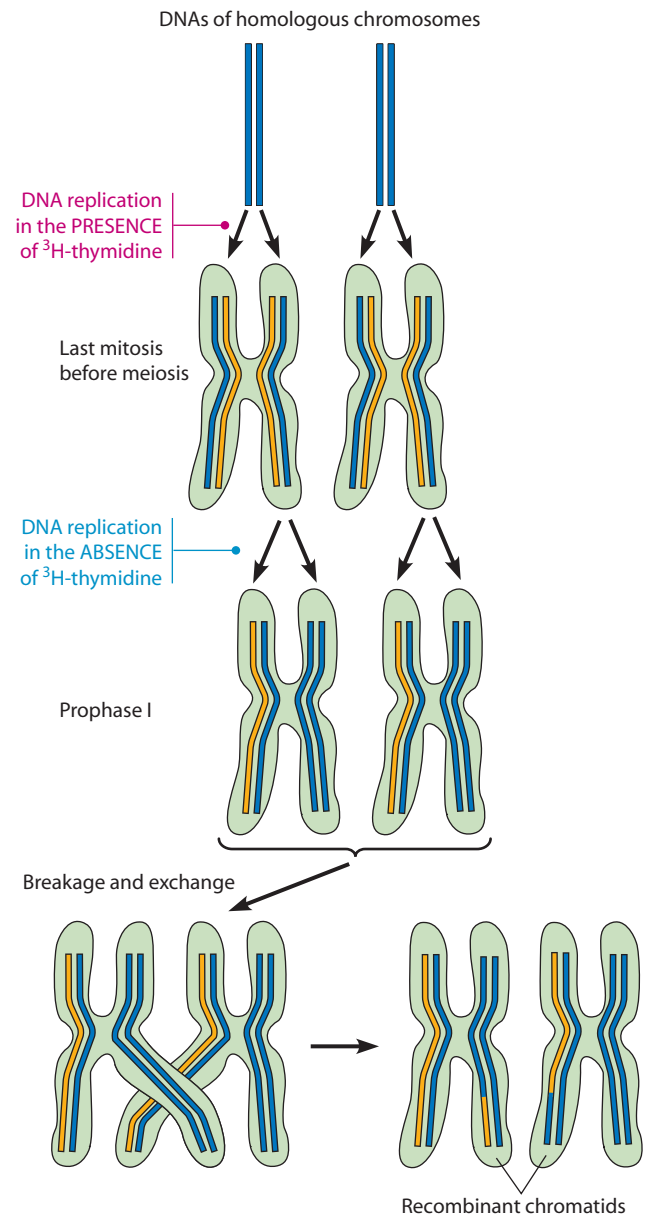


FIGURE 20-22 Experimental Demonstration of Breakage and Exchange During Eukaryotic Recombination. In this experiment, DNA was radioactively labeled by briefly exposing eukaryotic cells to ^{3}H-thymidine before the last mitosis prior to meiosis. When autoradiography was employed to examine the chromatids during meiosis, some were found to contain a mixture of labeled (orange) and unlabeled (dark blue) segments as predicted by the breakage-and-exchange model.

labeled chromatid and one unlabeled chromatid (**Figure 20-22**). But during the subsequent meiosis, individual chromatids exhibited a mixture of radioactive and nonradioactive segments, as would be predicted by the breakage-and-exchange model. Moreover, the frequency of such exchanges was directly proportional to the frequency with which the genes located in these regions underwent genetic recombination. Such observations provided strong support for the notion that genetic recombination in eukaryotic cells, as in prokaryotes, involves DNA breakage and exchange. These experiments also showed that most DNA exchanges arise between homologous chromosomes rather than between the two sister chromatids of a given chromosome. This selectivity is important because it ensures that genes are exchanged between paternal and maternal chromosomes.

Homologous Recombination Can Lead to Gene Conversion

The conclusion that homologous recombination is based on DNA breakage and exchange does not in itself provide much information concerning the underlying molecular mechanisms. One of the simplest breakage-and-exchange models that might be envisioned would involve the cleavage of two homologous, double-stranded DNA molecules at comparable locations, followed by exchange and rejoining of the cut ends. This model implies that genetic recombination should be completely reciprocal; that is, any genes exchanged from one chromosome should appear in the other chromosome and vice versa. For example, consider a hypothetical situation involving two genes designated *P* and *Q*. If one chromosome contains forms of these genes called *P1* and *Q1*, and the other chromosome has alternative forms designated *P2* and *Q2*, reciprocal exchange would be expected to

generate one chromosome with genes *P1* and *Q2* and a second chromosome with genes *P2* and *Q1*.

Although this reciprocal pattern is usually observed, recombination has been found to be nonreciprocal in some situations. For example, recombination might generate one chromosome with genes *P1* and *Q2* and a second chromosome with genes *P2* and *Q2*. In this particular example, the *Q1* gene expected on the second chromosome appears to have been converted to a *Q2* gene. For this reason, nonreciprocal recombination is often referred to as **gene conversion.** Gene conversion is most commonly observed when the recombining genes are located very close to one another. Because recombination between closely spaced genes is a rare event, gene conversion is most readily detectable in organisms that reproduce rapidly and generate large numbers of offspring, such as yeast and the common bread mold, *Neurospora*. More recently, however, gene conversion has been recognized in humans. For example, alteration of a gene by gene conversion underlies one form of *congenital adrenal hypoplasia*.

Homologous Recombination Is Initiated by Single-Strand DNA Exchanges (Holliday Junctions)

The preceding observations suggest that homologous recombination is more complicated than can be explained by a simple breakage-and-exchange model in which crossing over is accomplished by cleaving two double-stranded DNA molecules and then exchanging and rejoining the cut ends. Robin Holliday was the first to propose the alternative idea that recombination is based on the exchange of single DNA strands between two double-stranded DNA molecules. A current model of how such single-strand exchanges could lead to genetic recombination is illustrated in **Figure 20-23**. According to this model, the initial step (1) in recombination is the cleavage of one or both strands of the DNA double helix. In either case, a single broken DNA strand derived from one DNA molecule "invades" a complementary region of a homologous DNA double helix, displacing one of the two strands (2). Localized DNA synthesis and repair (3 and 4) ultimately generate a crossed structure, called a **Holliday junction,** in which a single strand from each DNA double helix has crossed over and joined the opposite double helix. Electron microscopy has provided direct support for the existence of Holliday junctions, revealing the presence of DNA double helices joined by single-strand crossovers at sites of genetic recombination (Figure 20-23, *inset*).

Once a Holliday junction has been formed, unwinding and rewinding of the DNA double helices causes the crossover point to move back and forth along the chromosomal DNA (5). This phenomenon, called *branch migration,* can rapidly increase the length of single-stranded DNA that is exchanged between two DNA molecules. After branch migration has occurred, the Holliday junction is cleaved and the broken DNA strands are rejoined to produce two separate DNA molecules. A Holliday junction can be cleaved and rejoined in either of two ways. If it is cleaved in one plane, the two DNA molecules that are produced will exhibit crossing over; that is, the chromosomal DNA beyond the point where recombination occurred will have been completely exchanged between the two chromosomes (6a). If the Holliday junction is cut in the other plane, crossing over does not occur, but the DNA molecules exhibit a noncomplementary region near the site where the Holliday junction had formed (6b).

What is the fate of such noncomplementary regions? If they remain intact, an ensuing mitotic division will separate the mismatched DNA strands; each strand will then serve as a template for the synthesis of a new complementary strand. The net result will be two new DNA molecules with differing base sequences and hence two cells containing slightly different gene sequences in the affected region. This is the situation occasionally observed in *Neurospora,* where two genetically different cells can arise during the mitosis following meiosis. Alternatively, a noncomplementary DNA region may be corrected by excision and repair. The net effect of DNA repair would be to convert genes from one form to another—in other words, gene conversion.

A key enzyme involved in homologous recombination was first identified using bacterial extracts that catalyze the formation of Holliday junctions. Such extracts contain a protein, called *RecA,* whose presence is required for recombination. Mutant bacteria that produce a defective RecA protein cannot carry out genetic recombination, nor are extracts prepared from such cells capable of creating Holliday junctions from homologous DNA molecules. The RecA protein catalyzes a *strand invasion* reaction in which a single-stranded DNA segment displaces one of the two strands of a DNA double helix; in other words, it catalyzes step 2 in Figure 20-23. Eukaryotes contain a comparable protein called *Rad51.* In catalyzing the strand invasion reaction, RecA or Rad51 first coats the single-stranded DNA region; the coated, single-stranded DNA then interacts with a DNA double helix, moving along the target DNA until it reaches a complementary sequence it can pair with.

Besides being involved in genetic recombination, strand invasion plays a role in certain types of DNA repair. As mentioned in Chapter 19, double-strand breaks in DNA are often repaired by a process that takes advantage of the fact that if one chromosome incurs a double-strand break, the intact DNA molecule of the homologous chromosomal DNA can serve as a template for guiding the repair of the broken chromosome. In such cases, single DNA strands from the broken DNA molecule invade the intact DNA molecule and find a complementary region to serve as a template for repairing the broken DNA, just as occurs in step 2 of Figure 20-23.

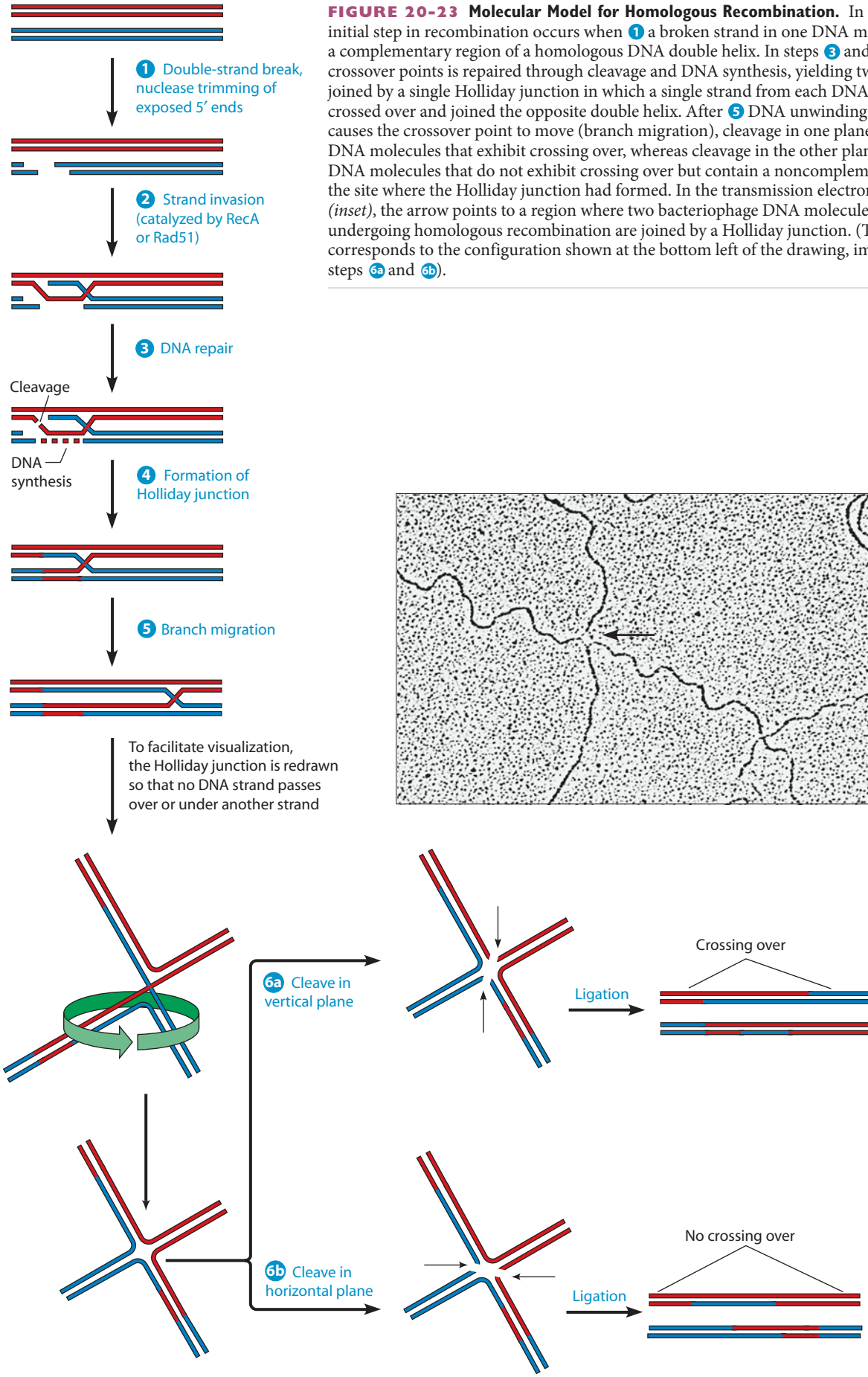


FIGURE 20-23 Molecular Model for Homologous Recombination. In this model, the initial step in recombination occurs when 1 a broken strand in one DNA molecule 2 invades a complementary region of a homologous DNA double helix. In steps 3 and 4, one of the crossover points is repaired through cleavage and DNA synthesis, yielding two DNA molecules joined by a single Holliday junction in which a single strand from each DNA double helix has crossed over and joined the opposite double helix. After 5 DNA unwinding and rewinding causes the crossover point to move (branch migration), cleavage in one plane 6a creates two DNA molecules that exhibit crossing over, whereas cleavage in the other plane 6b generates DNA molecules that do not exhibit crossing over but contain a noncomplementary region near the site where the Holliday junction had formed. In the transmission electron micrograph *(inset)*, the arrow points to a region where two bacteriophage DNA molecules in the process of undergoing homologous recombination are joined by a Holliday junction. (This photograph corresponds to the configuration shown at the bottom left of the drawing, immediately prior to steps 6a and 6b).

The Synaptonemal Complex Facilitates Homologous Recombination During Meiosis

Earlier in the chapter, we learned that during prophase I of meiosis, homologous chromosomes are joined together by a zipperlike, protein-containing structure called the synaptonemal complex. Several observations suggest that this structure plays an important role in genetic recombination. First, the synaptonemal complex appears at the time when recombination takes place. Second, its location between the opposed homologous chromosomes corresponds to the region where crossing over occurs. And finally, synaptonemal complexes are absent from organisms—such as male fruit flies—that fail to carry out meiotic recombination.

Presumably, the synaptonemal complex facilitates recombination by maintaining a close pairing between adjacent homologous chromosomes along their entire length. But if the synaptonemal complex facilitates recombination, how do cells ensure that such structures form only between homologous chromosomes? Evidence suggests the existence of a process called *homology searching,* in which a single-strand break in one DNA molecule produces a free strand that "invades" another DNA double helix and checks for the presence of complementary sequences (Figure 20-23, step ❷). If extensive homology is not found, the free DNA strand invades another DNA molecule and checks for complementarity, repeating the process until a homologous DNA molecule is detected. Only then does a synaptonemal complex develop, bringing the homologous chromosomes together throughout their length to facilitate the recombination process.

Recombinant DNA Technology and Gene Cloning

In nature, genetic recombination usually takes place between two DNA molecules derived from organisms of the same species. In animals and plants, for example, an individual's two parents are the original sources of the DNA that recombines during meiosis. A naturally arising recombinant DNA molecule usually differs from the parental DNA molecules only in the combination of alleles it contains; the fundamental identities and sequences of its genes remain the same.

In the laboratory, such limitations do not exist. Since the development of **recombinant DNA technology** in the 1970s, scientists have had at their disposal a collection of techniques for making recombinant DNA in the laboratory. Any segment of DNA can now be excised from any genome and spliced together with any other piece of DNA. Initially derived from basic research on the molecular biology of bacteria, these techniques have enabled researchers to isolate and study genes from any source with greater ease and precision than was earlier thought possible.

A central feature of recombinant DNA technology is the ability to produce specific pieces of DNA in large enough quantities for research and other uses. This process of generating many copies of specific DNA fragments is called **DNA cloning.** (In biology, a *clone* is a population of organisms that is derived from a single ancestor and hence is genetically homogeneous, and a *cell clone* is a population of cells derived from the division of a single cell. By analogy, a *DNA clone* is a population of DNA molecules that are derived from the replication of a single molecule and hence are identical to one another.)

DNA cloning is accomplished by splicing the DNA of interest to the DNA of a genetic element, called a **cloning vector,** that can replicate autonomously when introduced into a cell grown in culture—in most cases, a bacterium such as *E. coli*. The cloning vector can be a plasmid or the DNA of a virus, usually a bacteriophage; in either case, the vector's DNA "passenger" is copied every time it replicates. In this way, it is possible to generate large quantities of specific genes or other DNA segments—and of their protein products as well, if the passenger genes are transcribed and translated in proliferating cells that carry the vector.

To appreciate the importance of recombinant DNA technology, we need to grasp the magnitude of the problem that biologists faced as they tried to study the genomes of eukaryotic organisms. Much of our early understanding of information flow in cells came from studies with bacteria and viruses, whose genomes were mapped and analyzed in great detail using genetic methods that were not easily applied to eukaryotes. Until a few decades ago, investigators despaired of ever being able to understand and manipulate eukaryotic genomes to the same extent because the typical eukaryote has at least 10,000 times as much DNA as the best-studied phages—truly an awesome haystack in which to find a gene-sized needle. But the advent of recombinant DNA technology made it possible to isolate individual eukaryotic genes in quantities large enough to permit them to be thoroughly studied, ushering in a new era in biology.

The Discovery of Restriction Enzymes Paved the Way for Recombinant DNA Technology

Much of what we call recombinant DNA technology was made possible by the discovery of *restriction enzymes* (see Box 18B, page 520). The ability of restriction enzymes to cleave DNA molecules at specific sequences called *restriction sites* makes them powerful tools for cutting large DNA molecules into smaller fragments that can be recombined in various ways. Restriction enzymes that make staggered cuts in DNA are especially useful because they generate single-stranded *sticky ends* (also called *cohesive ends*) that provide a simple means for joining DNA fragments obtained from different sources. In essence, any two DNA fragments generated by the same restriction enzyme can be joined together by complementary base pairing between their single-stranded, sticky ends.

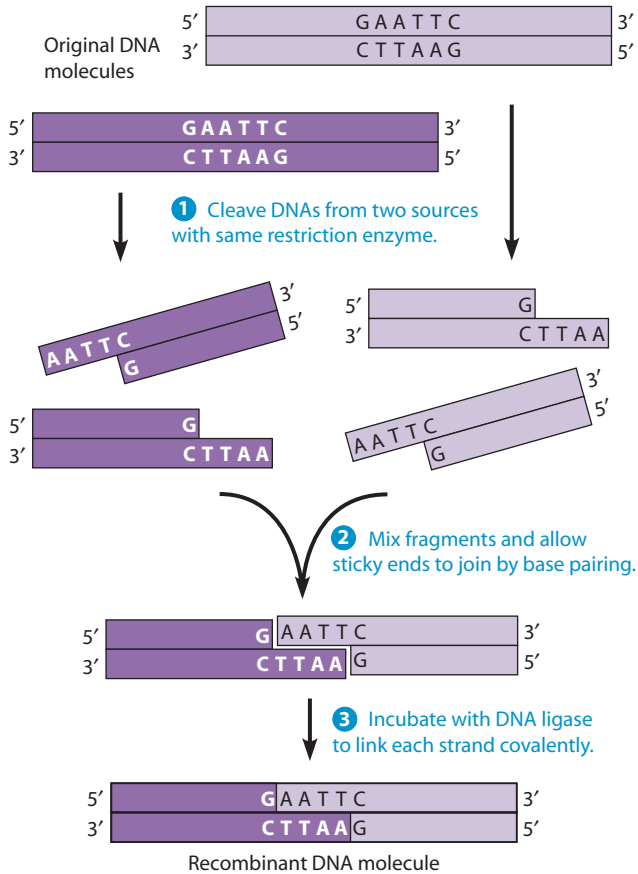


FIGURE 20-24 Creating Recombinant DNA Molecules. A restriction enzyme that generates sticky ends (in this case *Eco*RI) is used to cleave DNA molecules from two different sources. The complementary ends of the resulting fragments join by base pairing to create recombinant molecules containing segments from both of the original sources.

Figure 20-24 illustrates how this general approach works. DNA molecules from two sources are first treated with a restriction enzyme known to generate fragments with sticky ends (1), and the fragments are then mixed together under conditions that favor base pairing between these sticky ends (2). Once joined in this way, the DNA fragments are covalently sealed together by DNA ligase (3), an enzyme normally involved in DNA replication and repair (see Chapter 19). The final product is a **recombinant DNA molecule** containing DNA sequences derived from two different sources.

The combined use of restriction enzymes and DNA ligase allows any two (or more) pieces of DNA to be spliced together, regardless of their origins. A piece of human DNA, for example, can be joined to bacterial or phage DNA just as easily as it can be linked to another piece of human DNA. In other words, it is possible to form recombinant DNA molecules that never existed in nature, without regard for the natural barriers that otherwise limit recombination to genomes of the same or closely related species. Therein lies the power of (and, for some, the concern about) recombinant DNA technology.

DNA Cloning Techniques Permit Individual Gene Sequences to Be Produced in Large Quantities

The power of restriction enzymes is that they make it easy to insert a desired piece of DNA—usually a segment containing a specific gene—into a cloning vector that can replicate itself when introduced into bacterial cells. Suppose, for example, you wanted to isolate a gene that codes for a medically useful product, such as insulin needed for the treatment of diabetics or blood clotting factors needed by patients with hemophilia. By using restriction enzymes to insert a cDNA encoding such a protein (see the next section) into a cloning vector in bacterial cells and then identifying bacteria that contain the DNA of interest, it is possible to grow large masses of such cells and thereby obtain large quantities of the desired DNA (and its protein product).

Although the specific details vary, the following five steps are typically involved in this process of DNA cloning: (1) insertion of DNA into a cloning vector; (2) introduction of the recombinant vector into cultured cells, usually bacteria; (3) amplification of the recombinant vector in the bacteria; (4) selection of cells containing recombinant DNA; and (5) identification of clones containing the DNA of interest. **Figure 20-25** provides an overview of these events using a bacterial cloning vector. You should refer to this figure as we consider each step in turn.

1. Insertion of DNA into a Cloning Vector. The first step in cloning a desired piece of DNA is to insert it into an appropriate cloning vector, usually a bacteriophage or a plasmid. Most vectors used for DNA cloning are themselves recombinant DNA molecules, designed specifically for this purpose. For example, when bacteriophage λ DNA is used as a cloning vector, the phage DNA has had some of its nonessential genes removed to make room in the phage head for spliced-in DNA. Plasmids used as cloning vectors usually have a variety of restriction sites and often carry genes that confer antibiotic resistance on their host cells. The antibiotic-resistance genes facilitate the selection stage (4), while the presence of multiple kinds of restriction sites allows the plasmid to incorporate DNA fragments prepared with a variety of restriction enzymes.

Figure 20-26 illustrates the structure of *pUC19* ("puck-19"), a plasmid cloning vector developed in the mid-1980s. This plasmid, as well as a series of versatile vectors subsequently derived from it, carries a gene that confers resistance to the antibiotic ampicillin (amp^R). Bacteria containing such plasmids can therefore be identified by their ability to grow in the presence of ampicillin. The pUC19 plasmid also has 11 different restriction sites clustered in a region of the plasmid containing the *lacZ* gene, which codes for the enzyme β-galactosidase. Integration of foreign DNA at any of these restriction sites will disrupt the *lacZ* gene, thereby blocking the production of β-galactosidase. As we will see shortly, this

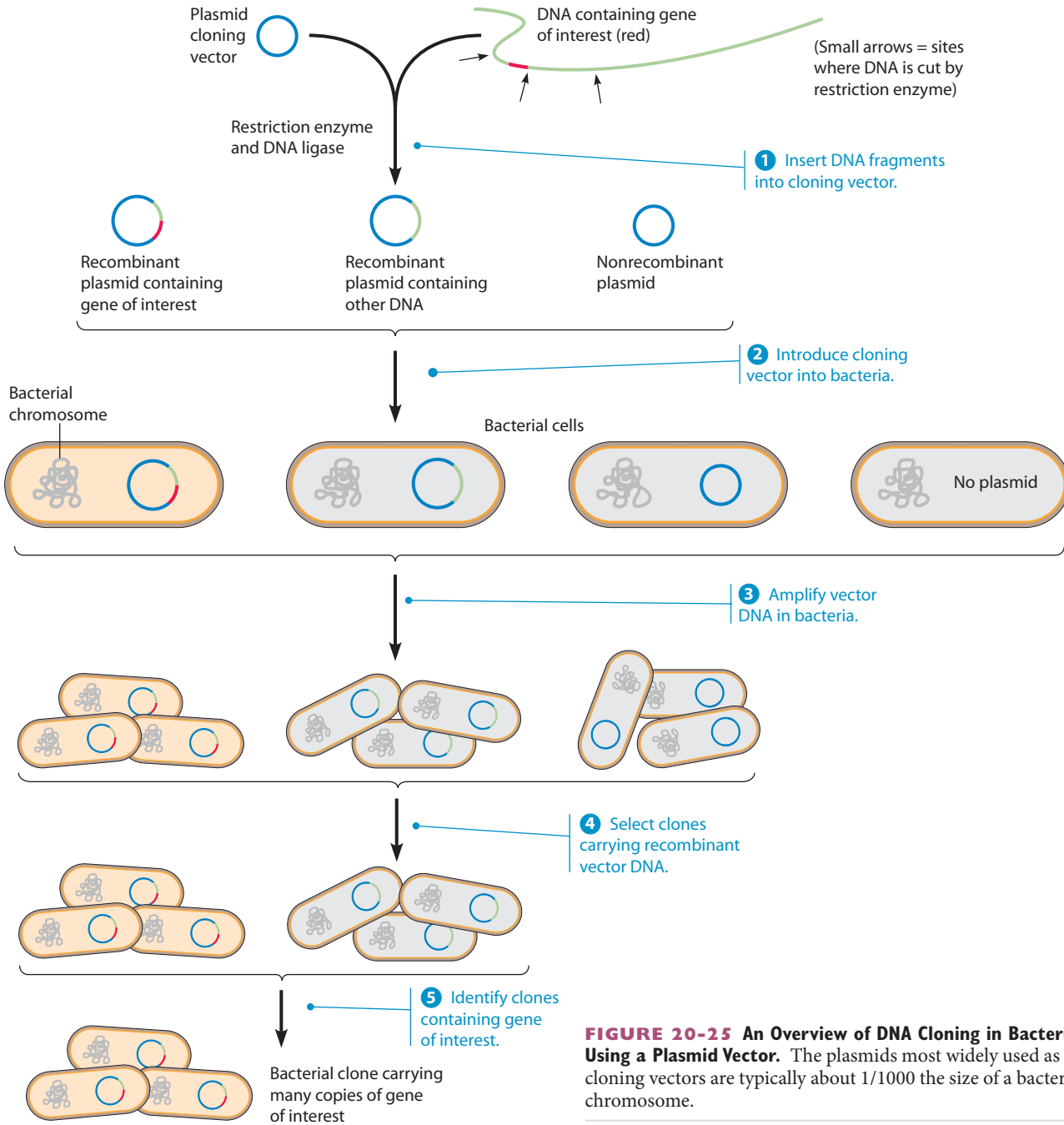


FIGURE 20-25 An Overview of DNA Cloning in Bacteria, Using a Plasmid Vector. The plasmids most widely used as cloning vectors are typically about 1/1000 the size of a bacterial chromosome.

disruption in β-galactosidase production can be used later in the cloning process to detect the presence of plasmids containing foreign DNA.

Figure 20-26b illustrates how a specific gene of interest residing in a foreign DNA source is inserted into a plasmid cloning vector, using pUC19 as the vector and a restriction enzyme that cleaves pUC19 at a single site within the *lacZ* gene. Incubation with the restriction enzyme cuts the plasmid at that site (❶), making the DNA linear (opening the circle). The same restriction enzyme is used to cleave the DNA molecule containing the gene to be cloned (❷). The sticky-ended fragments of foreign DNA are then incubated with the linearized vector molecules under conditions that favor base pairing (❸), followed by treatment with DNA ligase to link the molecules covalently (❹). To keep the diagram simple, Figure 20-26b shows only the recombinant plasmid containing the desired fragment of foreign DNA. In practice, however, a variety of DNA products will be present, including nonrecombinant plasmids and recombinant plasmids containing other fragments generated by the action of the restriction enzyme.

2. Introduction of the Recombinant Vector into Bacterial Cells. Once foreign DNA has been inserted into a cloning vector, the resulting recombinant vector is replicated

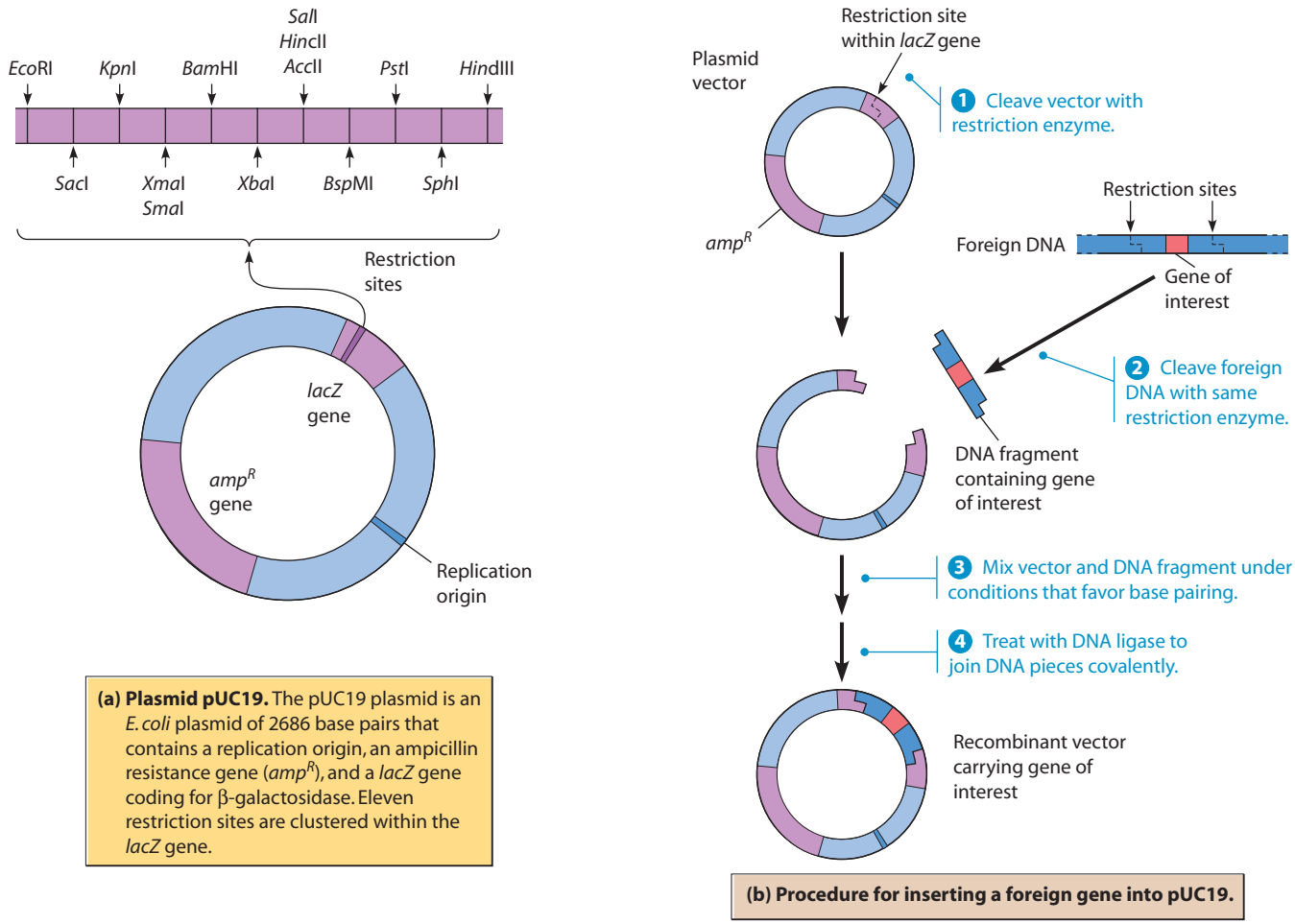


FIGURE 20-26 Cloning a Gene in the Plasmid Vector pUC19. To insert foreign DNA into the plasmid, the foreign and plasmid DNAs are cleaved with a restriction enzyme that recognizes the same site, in this case a site within the *lacZ* gene. The fragments of foreign DNA are incubated with the linearized plasmid DNA under conditions that favor base pairing between sticky ends. Among the expected products will be plasmid molecules recircularized by base pairing with a single fragment of foreign DNA, and some of these will contain the gene of interest. Cells carrying such plasmids will be resistant to ampicillin and will fail to produce β-galactosidase because of the foreign DNA inserted within the *lacZ* gene.

ACTIVITIES www.thecellplace.com *DNA cloning in a plasmid vector*

by introducing it into an appropriate host cell, usually the bacterium *E. coli.* Cloning vectors are introduced into bacteria in one of two ways. If the cloning vector is phage DNA, it is incorporated into phage particles that are then used to infect an appropriate cell population. Plasmids, on the other hand, are simply introduced into the medium surrounding the target cells. Both prokaryotic and eukaryotic cells will take up plasmid DNA from the external medium, although special treatments are usually necessary to enhance the efficiency of the process. Adding calcium ions, for example, markedly increases the rate at which cells take up DNA from the external environment.

3. Amplification of the Recombinant Vector in Bacteria. After they have taken up the recombinant cloning vector, the host bacteria are plated out on a nutrient medium so that the recombinant DNA vector can be replicated, or *amplified.* In the case of a plasmid vector, the bacteria proliferate and form colonies, each derived from a single cell. Under favorable conditions, *E. coli* will divide every 22 minutes, giving rise to a billion cells in less than 11 hours. As the bacteria multiply, the recombinant plasmids also replicate, producing an enormous number of vector molecules containing foreign DNA fragments. Under such conditions, a single recombinant plasmid introduced into one cell will be amplified several hundred billionfold in less than half a day.

In the case of phage vectors such as phage λ, a slightly different procedure is used. Phage particles containing recombinant DNA are mixed with bacterial cells, and the mixture is then placed on a culture medium under conditions that produce a continuous "lawn" of bacteria across the plate. Each time a phage particle infects a cell, the phage is replicated and eventually causes the cell to rupture and die. The released phage particles can then infect neighboring cells, repeating the process again. This

cycle eventually produces a clear zone of dead bacteria called a **plaque,** which contains large numbers of replicated phage particles derived by replication from a single type of recombinant phage (see Figure 18A-3). The millions of phage particles in each plaque contain identical molecules of recombinant phage DNA.

4. Selection of Cells Containing Recombinant DNA. During amplification of the cloning vector, procedures are introduced that preferentially select for the growth of those cells that have successfully incorporated the vector. For plasmid vectors such as pUC19, the selection method is based on the plasmid's antibiotic-resistance genes. For example, all bacteria carrying the recombinant plasmids generated in Figure 20-26b will be resistant to the antibiotic ampicillin, since all plasmids have an intact ampicillin-resistance gene. The amp^R gene is a **selectable marker,** which allows only the cells carrying plasmids to grow on culture medium containing ampicillin (the medium "selects for" the growth of the ampicillin-resistant cells).

However, not all the ampicillin-resistant bacteria will carry *recombinant* plasmids—that is, plasmids containing spliced-in DNA. But those bacteria that do contain recombinant plasmids can be readily identified because the *lacZ* gene has been disrupted by the foreign DNA and, as a result, β-galactosidase will no longer be produced. The lack of β-galactosidase can be detected by a simple color test in which bacteria are exposed to a substrate that is normally cleaved by β-galactosidase into a blue-staining compound. Bacterial colonies containing the normal pUC19 plasmid will therefore stain blue, whereas colonies containing recombinant plasmids with inserted DNA fragments will appear white.

A different approach is used with phage cloning vectors, which are usually derived from phage λ DNA molecules that are only about 70% as long as normal phage DNA. As a result, these DNA molecules are too small to be packaged into functional phage particles. But if an additional fragment of DNA is inserted into the middle of such a cloning vector, it creates a recombinant DNA molecule that is larger and thus capable of being assembled into a functional phage (**Figure 20-27**). Hence, when phage cloning vectors are employed, the only phage particles that can successfully infect bacterial cells are those containing an inserted foreign DNA sequence.

5. Identification of Clones Containing the DNA of Interest. The preceding steps typically generate vast numbers of bacteria producing many different kinds of recombinant DNA, only one or a few of which are relevant to the desired application. The final stage in any recombinant DNA procedure is therefore screening the bacterial colonies (or phage plaques) to identify those containing the specific DNA fragment of interest. For standard bacterial clones, this is usually done by isolating DNA from the bacteria and then using restriction enzymes to confirm that the cloned DNA has the expected pattern. The cloned DNA is then often sequenced (see page 520) to provide precise verification of the nature of the inserted DNA.

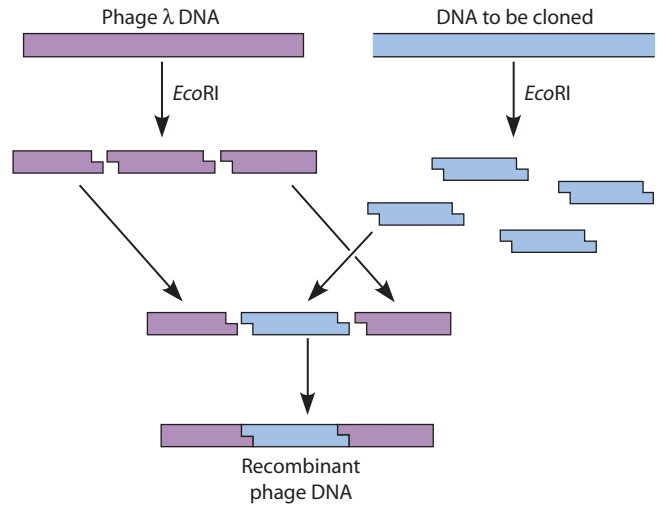


FIGURE 20-27 Bacteriophage λ as a Cloning Vector. The middle segment of the phage λ DNA molecule is removed by *Eco*RI cleavage and then replaced by the DNA fragment to be cloned. The inserted DNA fragment is necessary to make the phage λ DNA molecule large enough to be packaged into a functional phage particle.

Genomic and cDNA Libraries Are Both Useful for DNA Cloning

Cloning foreign DNA in bacterial cells is now a routine procedure. In practice, obtaining a good source of DNA to serve as starting material is often one of the most difficult steps. Two different approaches are commonly used for producing the DNA starting material. In the "shotgun" approach, an organism's entire genome (or some substantial portion of it) is cleaved into a large number of restriction fragments, which are then inserted into cloning vectors for introduction into bacterial cells (or phage particles). The resulting group of clones is called a **genomic library** because it contains cloned fragments representing most, if not all, of the genome. Genomic libraries of eukaryotic DNA are valuable resources from which specific genes can be isolated, provided that a sufficiently sensitive identification technique is available. Once a rare bacterial colony containing the desired DNA fragment has been identified, it can be grown on a nutrient medium to generate as many copies of the fragment as may be needed. Of course, the DNA cuts made by a restriction enzyme do not respect gene boundaries, and some genes may be divided among two or more restriction fragments. This problem can be circumvented by carrying out a *partial DNA digestion* in which the DNA is briefly exposed to a small quantity of restriction enzyme. Under such conditions, some restriction sites remain uncut, increasing the probability that at least one intact copy of each gene will be present in the genomic library.

The alternative DNA source for cloning experiments is DNA that has been generated by copying messenger RNA (mRNA) with the enzyme *reverse transcriptase* (page 648). This reaction generates a population of **complementary DNA (cDNA)** molecules that are complementary in sequence to the mRNA employed as template (**Figure 20-28**). If the entire mRNA population of a cell is isolated and copied into cDNA for cloning, the resulting group of clones is called a **cDNA library.** The advantage of a cDNA library is that it contains only those DNA sequences that are transcribed into mRNA—presumably, the active genes in the cells or tissue from which the mRNA was prepared.

Besides being limited to transcribed genes, a cDNA library has another important advantage as a starting point for the cloning of eukaryotic genes. Using mRNA to make cDNA guarantees that the cloned genes will contain only gene-coding sequences, without the noncoding interruptions called *introns* that are common in eukaryotic genes (see Chapter 21). Introns can be so extensive that the overall length of a eukaryotic gene becomes too unwieldy for recombinant DNA manipulation. Using cDNA eliminates this problem. In addition, bacteria cannot synthesize the correct protein product of an intron-containing eukaryotic gene unless the introns have been removed—as they are in cDNA.

Once a DNA library has been constructed, how can bacteria or phage be identified that carry only the sequences that we are interested in? There are several techniques for *screening* DNA libraries. The particular technique used depends on what the researcher knows about the gene being cloned, and on the type of library. If something is known about the base sequence of the gene of interest, the researcher can employ a **nucleic acid probe,** a single-stranded molecule of DNA or RNA that can identify a desired DNA sequence by base-pairing with it. Nucleic acid probes are labeled either with radioactivity or with some other chemical group that allows the probe to be easily visualized. (In Box 18C, page 528, we saw such a probe used to identify restriction fragment bands in Southern blotting.) The researcher prepares a labeled DNA or RNA probe containing all or part of the nucleotide sequence of interest and uses it to tag the colonies that contain complementary DNA. **Figure 20-29** outlines how this *colony hybridization technique* can be used to screen for colonies carrying the desired DNA. Once the appropriate colonies have been identified, cloned DNA is recovered from these colonies by isolating the vector DNA from the bacterial cells and digesting it with the same restriction enzyme used initially.

Another screening approach focuses on the protein encoded by a gene of interest. If this protein is known and has been purified, antibodies against it can be prepared and used as probes to check bacterial colonies for the presence of the protein. Alternatively, the *function* of the protein can be measured; for example, an enzyme could be tested for its catalytic activity. Protein-screening methods obviously depend on the ability of the bacterial cells to produce a foreign protein encoded by a cloned gene and will fail to detect cloned genes that are not expressed in the host cell. However, special *expression vectors* can be used to increase the likelihood that bacteria will transcribe eukaryotic genes properly and in large amounts. Expression vectors contain special DNA sequences that signal the bacterial cell to perform these processes.

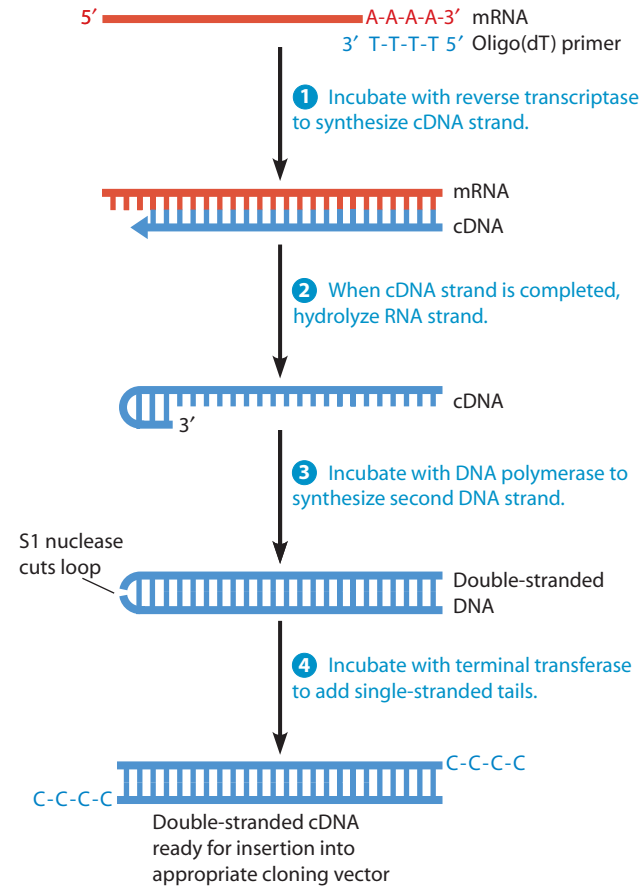


FIGURE 20-28 Preparation of Complementary DNA (cDNA) for Cloning. 1 Messenger RNA is incubated with reverse transcriptase to create a complementary DNA (cDNA) strand. Oligo(dT), a short chain of thymine deoxynucleotides, can be used as a primer because eukaryotic mRNA always has a stretch of adenine nucleotides at its 3′ end. 2 The resulting mRNA-cDNA hybrid is treated with alkali or an enzyme to hydrolyze the RNA, leaving the single-stranded cDNA. 3 DNA polymerase can now synthesize the complementary DNA strand, using the looped-around 3′ end of the first DNA strand as a primer. An enzyme called S1 nuclease is then employed to cleave the loop. 4 For efficient insertion into a cloning vector, the double-stranded DNA must have single-stranded tails that are complementary to those of the vector. These can be added by incubating with terminal transferase, an enzyme that adds nucleotides to DNA ends. For example, if short stretches of cytosine (C) nucleotides are added to the cDNA and short stretches of guanine (G) nucleotides are added to a linearized cloning vector, recombinant molecules can be generated by allowing the single-stranded C tails in the cDNA to hybridize to the single-stranded G tails in the vector. (As an alternative to step 4, short synthetic "linker" molecules containing a variety of restriction sites can be ligated to the ends of both the cDNA and a blunt-ended cloning vector. The linkers are then cleaved with a restriction enzyme that generates sticky ends.)

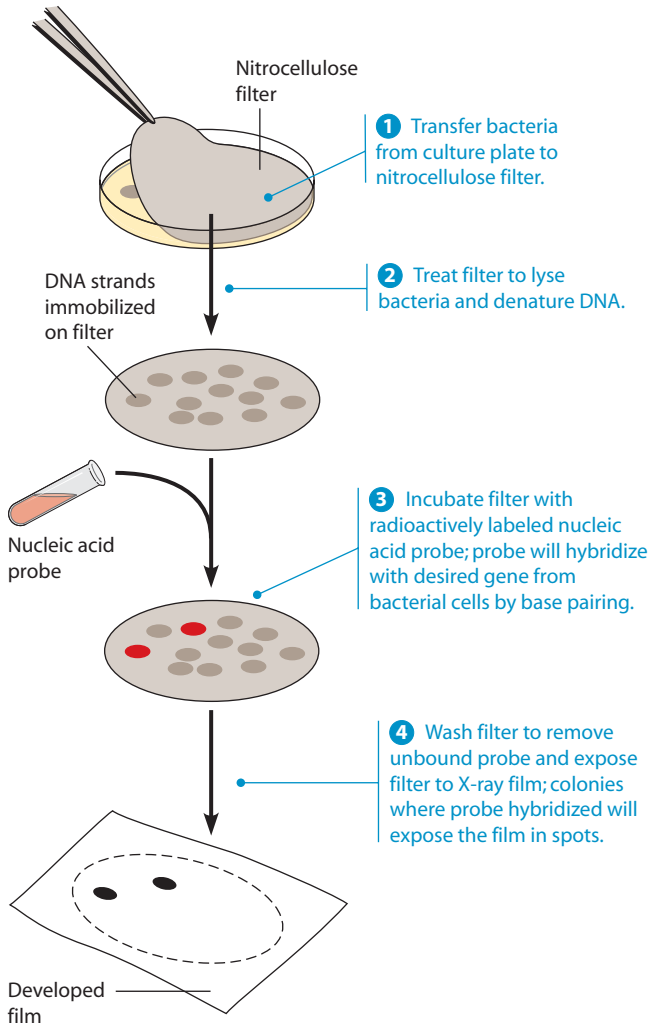


FIGURE 20-29 Colony Hybridization Technique. This technique is used to screen bacterial colonies for the presence of DNA that is complementary in sequence to a nucleic acid probe. Bacterial colonies are transferred from the surface of an agar culture plate onto a nitrocellulose filter, which is treated with detergent to lyse the bacteria and alkali (NaOH) to denature their DNA. The filters are then incubated with molecules of the nucleic acid probe—radioactively labeled, single-stranded DNA or RNA—which attach by base pairing to any complementary DNA present on the filter. The filter is rinsed and subjected to autoradiography, which makes visible those colonies containing DNA that is complementary in sequence to the probe.

Large DNA Segments Can Be Cloned in YACs and BACs

DNA cloning using the vectors mentioned so far is a powerful methodology, but it has an important limitation: The foreign DNA fragments cloned in these vectors cannot exceed about 30,000 base pairs (bp) in length. Eukaryotic genes are often larger than this and hence cannot be cloned in an intact form using such vectors. For genome-mapping projects, the availability of clones containing even longer stretches of DNA is desirable because the more DNA per clone, the fewer the number of clones needed to cover the entire genome.

One of the first breakthroughs in cloning longer DNA segments was the development of a vector called a **yeast artificial chromosome (YAC).** A YAC is a "minimalist" eukaryotic chromosome—it contains all the DNA sequences needed for normal chromosome replication and segregation to daughter cells–and very little else. As you might guess from your knowledge of chromosome replication and segregation (see Chapter 19), a eukaryotic chromosome requires three kinds of DNA sequences: (1) an origin of DNA replication; (2) two telomeres to allow periodic extension of the shrinking ends by telomerase; and (3) a centromere to ensure proper attachment, via a kinetochore, to spindle microtubules during cell division. If yeast versions of these three kinds of DNA sequences are combined with a segment of foreign DNA, the resulting YAC will replicate in yeast and segregate into daughter cells with each round of cell division, just like a natural chromosome. And under appropriate conditions, its foreign genes may be expressed.

Figure 20-30 outlines the construction of a typical YAC. In addition to a replication origin (ORI), centromere sequence (CEN), and two telomeres (TEL), the vector illustrated carries two genes that function as selectable markers as well as three restriction sites. In cloning experiments, the vector and foreign DNAs are cleaved with the appropriate restriction enzymes, mixed together, and joined by DNA ligase. The resulting products, which include a variety of YACs carrying different fragments of foreign DNA, are introduced into yeast cells whose cell walls have been removed. The presence of two selectable markers makes it easy to select for yeast cells containing YACs with both chromosomal "arms." The diagram in the figure is not to scale: The YAC vector alone is only about 10,000 bp, but the inserted foreign DNA usually ranges from 300,000 to 1.5 million bp in length. In fact, YACs must carry at least 50,000 bp to be reliably replicated and segregated.

Another type of vector used for cloning large DNA fragments is the **bacterial artificial chromosome (BAC),** a derivative of the *F factor* plasmid that some bacteria employ for transferring DNA between cells during bacterial conjugation (page 621). BAC vectors are modified forms of the F factor plasmid that can hold up to 350,000 bp of foreign DNA and have all the components required for a bacterial cloning vector, such as replication origins, antibiotic resistance genes, and insertion sites for foreign DNA. One type of BAC facilitates the process of screening for recombinant clones by including the *SacB* gene, which converts sucrose (table sugar) into a substance that is toxic to bacteria. A *Bam*HI cloning site is located within the *SacB* gene, so when foreign DNA is inserted into the BAC vector at this site, the *SacB* gene is disrupted. When such a BAC vector is introduced into bacterial cells grown in the presence of sucrose, only cells containing BAC vector molecules with a foreign DNA insert will be able to grow. Those cells receiving BAC vector with no DNA insert will fail to grow because the *SacB* gene remains intact and produces a toxic substance from sucrose.

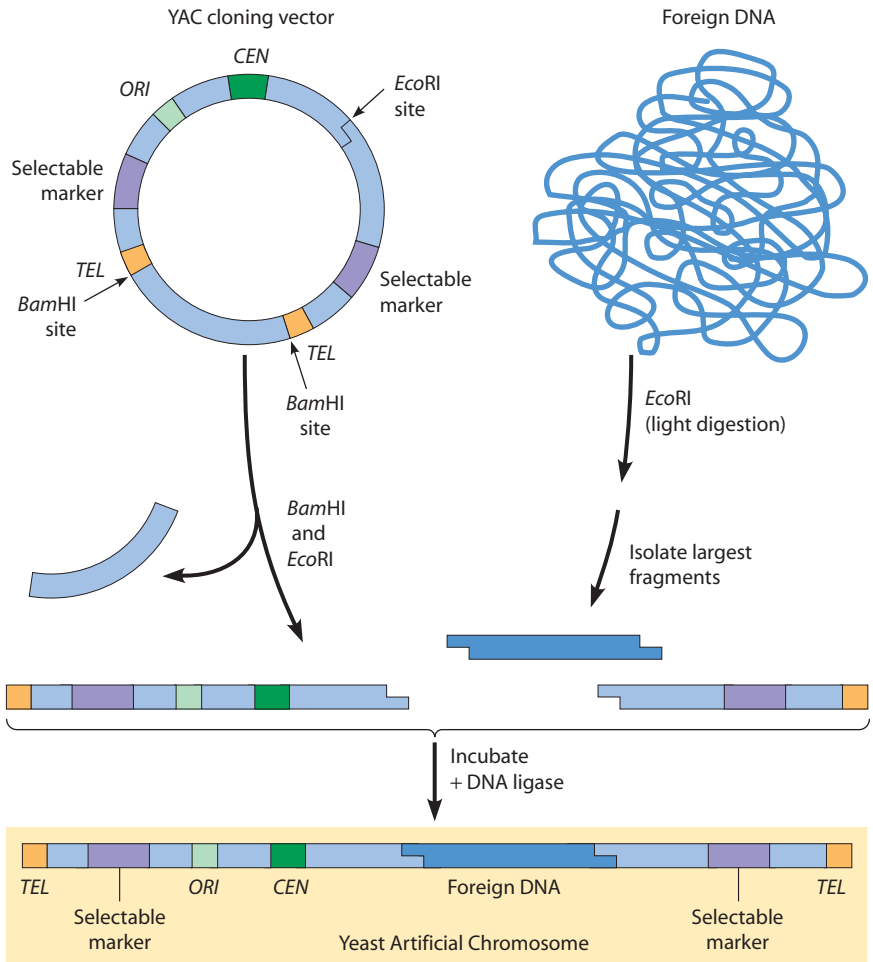


FIGURE 20-30 Construction of a Yeast Artificial Chromosome (YAC). The YAC cloning vector is a circular DNA molecule with nucleotide sequences specifying an origin of DNA replication *(ORI)*, a centromere *(CEN)*, two telomeres *(TEL)*, and two selectable markers. It has two recognition sequences for the restriction enzyme *Bam*HI and one for *Eco*RI. Digestion of the YAC vector with both restriction enzymes produces two linear DNA fragments that together contain all the essential sequences, as well as the fragment that connected the *Bam*HI sites, which is of no further use. The fragment mixture is incubated with fragments from light digestion of foreign DNA with *Eco*RI (light digestion generates large DNA fragments because not all the restriction sites are cut), and the resulting recombinant strands are sealed with DNA ligase. Among the products will be YACs carrying foreign DNA, as shown at the bottom of the figure. After yeast cells are transformed with the products of the procedure, the colonies of cells that have received complete YACs can be identified by the properties conferred by the two selectable markers.

PCR Is Widely Used to Clone Genes from Sequenced Genomes

For many years the cloning procedures described in the preceding sections were the primary means for producing cloned genes and providing longer stretches of DNA for genome sequencing. But now that scientists have determined the genome sequences of hundreds of bacteria and several dozen eukaryotic organisms, including humans, the simpler and quicker *polymerase chain reaction (PCR)* method is more commonly used to clone genes from cDNA or genomic DNA libraries. As described in Box 19A on page 560, the PCR method simply requires that you know part of the base sequence of the gene you wish to amplify. The next step is to synthesize short, single-stranded DNA primers that are complementary to sequences located at opposite ends of the gene; these primers are then used to target the intervening DNA for amplification.

In addition to its speed and simplicity, another advantage of PCR is that it allows genes to be modified by adding desired base sequences to the primers being used. One such modification, called *epitope tagging,* involves adding a short base sequence coding for a stretch of amino acids that is recognized by a commercially available antibody. When a cloned gene produced in this way is expressed inside cells, the protein produced by the gene contains the additional stretch of amino acids and can therefore be visualized and tracked using the antibody attached to a fluorescent dye. Another type of modification, called *polyhistidine tagging* (or *His* tagging, for short), introduces a short base sequence coding for the amino acid histidine repeated six times in succession. The presence of six adjacent histidines in a protein creates a structure that selectively binds to nickel ions, allowing the protein to be rapidly purified based on its affinity for nickel.

Genetic Engineering

Recombinant DNA technology has had an enormous impact on the field of cell biology, leading to many new insights into the organization, behavior, and regulation of genes and their protein products. Many of these discoveries would have been virtually inconceivable without such powerful techniques for isolating gene sequences. The rapid advances in our ability to manipulate genes have also opened up the field of **genetic engineering,** which involves the application of recombinant DNA technology to practical problems, primarily in medicine and agriculture. In concluding the chapter, we will briefly examine some of the areas where these practical

benefits of recombinant DNA technology are beginning to be seen.

Genetic Engineering Can Produce Valuable Proteins That Are Otherwise Difficult to Obtain

One practical benefit to emerge from recombinant DNA technology is the ability to clone genes coding for medically useful proteins that are difficult to obtain by conventional means. Among the first proteins to be produced by genetic engineering was human *insulin,* which is required by roughly 2 million diabetics in the United States to treat their disease. Supplies of insulin purified from human blood or pancreatic tissue are extremely scarce; thus, for many years diabetic patients were treated with insulin obtained from pigs and cattle, which can cause toxic reactions. Now there are several ways of producing human insulin from genetically engineered bacteria containing the human insulin gene. As a result, diabetics can be treated with insulin molecules that are identical to the insulin produced by the human pancreas.

Like insulin, a variety of other medically important proteins that were once difficult to obtain in adequate amounts are now produced using recombinant DNA technology. Included in this category are the *blood-clotting factors* needed for the treatment of hemophilia, *growth hormone* utilized for treating pituitary dwarfism, *tissue plasminogen activator (TPA)* used for dissolving blood clots in heart attack patients, *erythropoietin* employed for stimulating the production of red blood cells in patients with anemia, and *tumor necrosis factor, interferon,* and *interleukin,* which are used in treating certain kinds of cancer. Traditional methods for isolating and purifying such proteins from natural sources are quite cumbersome and tend to yield only tiny amounts of protein. Thus, before the advent of recombinant DNA technology, the supplies of such substances were inadequate and their cost was extremely high. But now that the genes for these proteins have been cloned in bacteria and yeast, large quantities of protein can be produced in the laboratory at reasonable cost.

The Ti Plasmid Is a Useful Vector for Introducing Foreign Genes into Plants

Recombinant DNA technology is also being used to modify agriculturally important plants by inserting genes designed to introduce traits such as resistance to insects, herbicides, or viral disease, or to improve a plant's nitrogen-fixing ability, photosynthetic efficiency, nutritional value, or ability to grow under adverse conditions. Cloned genes are transferred into plants by inserting them first into the **Ti plasmid,** a naturally occurring DNA molecule carried by the bacterium *Agrobacterium tumefaciens.* In nature, infection of plant cells by this bacterium leads to insertion of a small part of the plasmid DNA, called the *T DNA region,* into the plant cell chromosomal DNA; expression of the inserted DNA then triggers the formation of an uncontrolled growth of tissue called a *crown gall tumor.* In the laboratory, the DNA sequences that trigger tumor formation can be removed from the Ti plasmid without stopping the transfer of DNA from the plasmid to the host cell chromosome. Inserting genes of interest into such modified plasmids produces vectors that can transfer foreign genes into plant cells.

The general approach for transferring genes into plants using these plasmids is summarized in **Figure 20-31**. The desired foreign gene is first inserted into the T DNA region of the isolated plasmid using standard recombinant DNA techniques, the plasmid is put back into the *Agrobacterium* bacteria, and these genetically engineered bacteria are then used to infect plant cells growing in culture. When the recombinant plasmid enters the plant cell, its T DNA becomes stably integrated into the plant genome and is passed on to both daughter cells at every cell division. Such cells are subsequently used to regenerate plants that contain the recombinant T DNA—and therefore the desired foreign gene—in all of their cells. The foreign gene will now be inherited by progeny plants just like any other gene. Such plants are said to be **transgenic,** a general term that refers to any type of organism, plant or animal, that carries one or more genes from another organism in all of its cells, including its reproductive cells. Transgenic plants are also commonly referred to as *GM (genetically modified)* plants.

Genetic Modification Can Improve the Traits of Food Crops

The ability to insert new genes into plants using the Ti plasmid has allowed scientists to create GM crops exhibiting a variety of new traits. For example, plants can be made more resistant to insect damage by introducing a gene cloned from the soil bacterium *Bacillus thuringiensis (Bt).* This *Bt* gene codes for a protein that is toxic to certain insects—especially caterpillars and beetles that cause crop damage by chewing on plant leaves. Putting the *Bt* gene into plants such as cotton and corn has permitted farmers to limit their use of more hazardous pesticides for controlling insects, leading to improved crop yields and a significant return of wildlife to crop fields. When several million cotton farmers in China switched to growing such an insect-resistant strain of GM cotton, it allowed them to slash pesticide use up to 70%. This success was accompanied by increased cotton yields and a significant drop in the death toll among farmworkers from pesticide poisoning. Similar economic and health benefits have been reported by some farmers growing insect-resistant strains of GM rice. Crops engineered to resist weed-killing herbicides likewise require fewer toxic chemicals and exhibit higher yields.

Another goal of genetic modification is to improve the nutritional value of food. Consider rice, for example, which is the most common food source in the world. More than 3 billion people currently eat rice daily, and by the year 2020 at least half the world's population is

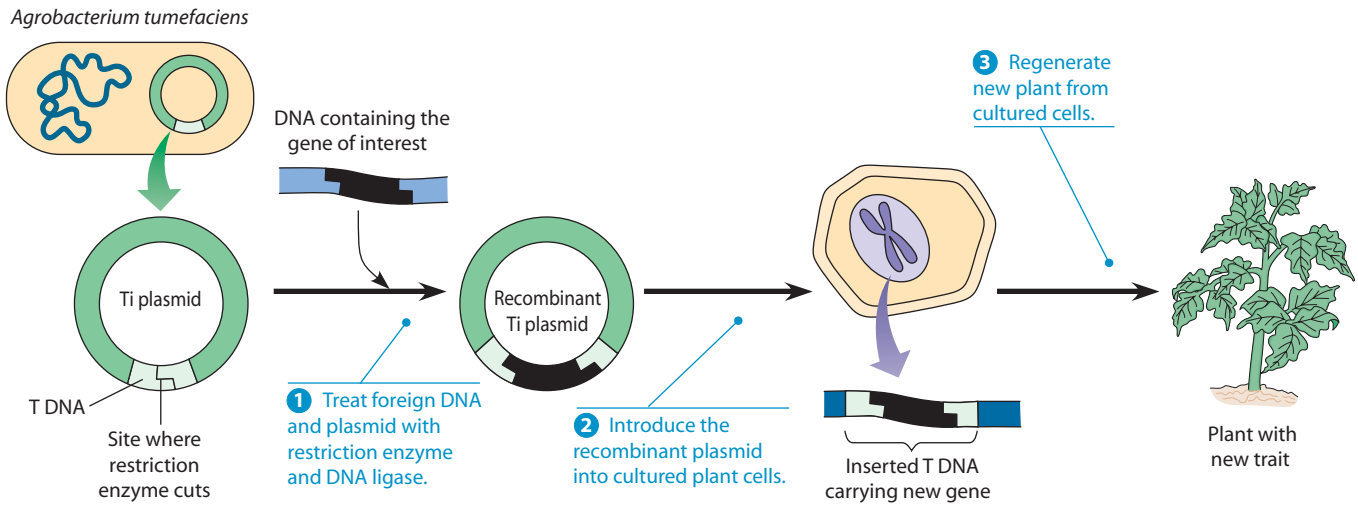


FIGURE 20-31 Using the Ti Plasmid to Transfer Genes into Plants. Most genetic engineering in plants uses the Ti plasmid as a vector. A DNA fragment containing a gene of interest is inserted into a restriction site located in the T DNA region of the plasmid. The recombinant plasmid is then introduced into plant cells, which regenerate a new plant containing the recombinant T DNA stably incorporated into the genome of every cell.

expected to depend on rice for food. Such dependence will be especially prevalent in poor, developing countries, where much of the population also suffers from deficiencies in essential nutrients and vitamins. To illustrate how genetic engineering might be used to improve the situation, the genes required for the synthesis of β-carotene, a precursor of vitamin A, were genetically engineered into rice in 2001, and the β-carotene content of such rice was increased more than 20-fold a few years later. The resulting product, called "golden rice" because of the color imparted by β-carotene, has the potential to help alleviate a global vitamin A deficiency that now causes blindness and disease in millions of children.

Concerns Have Been Raised About the Safety and Environmental Risks of GM Crops

As the prevalence of GM crops has begun to increase, some people have expressed concerns about the possible risks associated with this technology, especially because it permits genes to cross species barriers that cannot be crossed by traditional breeding techniques. For consumers, the main focus has been on safety because it is well known that toxic and allergic reactions to things we eat can be serious and even life-threatening. Thus far, it seems clear that conventional foods already on the market, such as peanuts and Brazil nuts, pose greater allergy risks than have been demonstrated for any GM foods. Moreover, the same problem also arises when new crop strains are produced by conventional breeding techniques. In fact, conventional foods have already undergone massive changes in genetic makeup by traditional plant-breeding methods, and it can be argued that it is safer to insert carefully selected genes into plants one at a time, as is done in genetic engineering, than it is to alter thousands of genes at a time, as is done with traditional crossbreeding of plants. When a single gene is inserted into a crop, the effects of that single gene and its protein product can be more easily assessed for safety hazards.

The possibility that GM crops may pose environmental risks has also raised concerns. In a 1999 laboratory experiment, scientists reported that monarch butterfly larvae died after being fed leaves dusted with pollen from GM corn containing the *Bt* gene. This observation led to fears that the *Bt* toxin produced by GM plants can harm friendly insects. However, the lab bench is not the farm field, and the initial studies were carried out under artificial laboratory conditions in which butterfly larvae consumed far higher doses of *Bt* toxin than they would in the real world. Subsequent data collected from farm fields containing GM crops suggest that the amount of *Bt* corn pollen encountered under real-life conditions does not pose a significant hazard to monarch butterflies.

Despite legitimate concerns about potential hazards, the GM experience has thus far revealed little evidence of significant risks to either human health or the environment. Of course, no new technology is entirely without risk, and so the safety and environmental impact of GM crops must be continually assessed. At the same time, GM crops have allowed reductions in pesticide use, and they could make a unique contribution to the fight against hunger and disease in developing countries.

Animals Can Be Genetically Modified by Adding or Knocking Out Specific Genetic Elements

Just as we have seen with plants, it is possible to genetically engineer animals. The techniques by which engineered DNA is introduced varies among different animals but often includes microinjection of engineered DNA into an

adult animal or embryo. One of the first successful demonstrations of the feasibility of gene transplantation in animals was reported by Richard Palmiter and Ralph Brinster, who transferred the gene for growth hormone into a fertilized mouse egg, thereby creating a transgenic mouse that carries a gene from another organism in its cells (**Box 20A**). Transgenic animals have been extremely useful in several ways. In the laboratory, genetic engineering has allowed the study of gene expression and the function of specific genes in living animals. For example, when proteins are engineered so that they are fused with the green fluorescent protein (GFP), their location can be followed dynamically in living cells (see page 7 and the Appendix, page A-10).

In addition to their usefulness in basic research, there are practical applications of genetic engineering in animals. One goal of such technology is to produce farm animals that can synthesize medically important human proteins (e.g., in the milk of female mammals), which can then be easily purified. Another is the production of engineered livestock as a food source. Many of the same issues we considered regarding GM crops have also been raised concerning genetically modified animals grown for food.

While adding an engineered piece of DNA to an animal using transgenic technology can be very useful, it is often even more useful to *remove* a gene of interest. One way in which this can be done exploits the ability of homologous recombination to transfer base sequences between related DNA molecules. For example, scientists can now inactivate or "knock out" individually targeted genes by inducing recombination with related pieces of nonfunctional DNA (**Figure 20-32**). This strategy has been used to create hundreds of unique strains of **knockout mice,** each defective in a single gene, and efforts are under way to create knockout strains for every one of the mouse's roughly 25,000 genes. In 2007 Mario Capecchi, Oliver Smithies, and Martin Evans were awarded a Nobel Prize in recognition of their pioneering efforts in making the creation of knockout mice possible.

The first step in producing a knockout mouse is to synthesize an artificial DNA that is similar in base sequence to the target gene and its flanking sequences but with two important changes (Figure 20-32, ❶). First, an antibiotic resistance gene (e.g., that confers resistance to neomycin) is inserted into the middle of the target gene sequence. This simultaneously renders the engineered copy of the gene nonfunctional and also allows cells that carry the DNA to survive in the presence of antibiotic. Second, DNA encoding a viral enzyme, *thymidine kinase,* is attached to the end of the DNA. If this DNA is present in a cell, it will die in the presence of an antiviral drug (e.g., ganciclovir). Next this DNA is introduced into mouse *embryonic stem cells (ES cells),* which are cells that can differentiate into all the cell types of an adult mouse (❷). In very rare cases, the DNA enters the nucleus and, using mechanisms similar to those involved in meiosis, the artificial DNA aligns with complementary sequences flanking the targeted gene. Homologous recombination then replaces the targeted gene with the new, nonfunctional copy. Only if homologous recombination occurs is the thymidine kinase gene removed from the engineered DNA and degraded by nucleases in the ES cell. In this case, the cell will survive in the presence of antibiotic (❸), but it will *not* be sensitive to the antiviral drug (❹). Cells identified using this double drug selection are then introduced into mouse embryos, which develop into adult mice containing tissues where the gene of interest has been inactivated (❺). Crossbreeding such animals eventually yields strains of pure knockout mice in which both copies of the target gene (one on each chromosome) are knocked out in all tissues (❻). By allowing scientists to study what happens when specific genes are disrupted, knockout mice have shed light on the roles played by individual genes in numerous

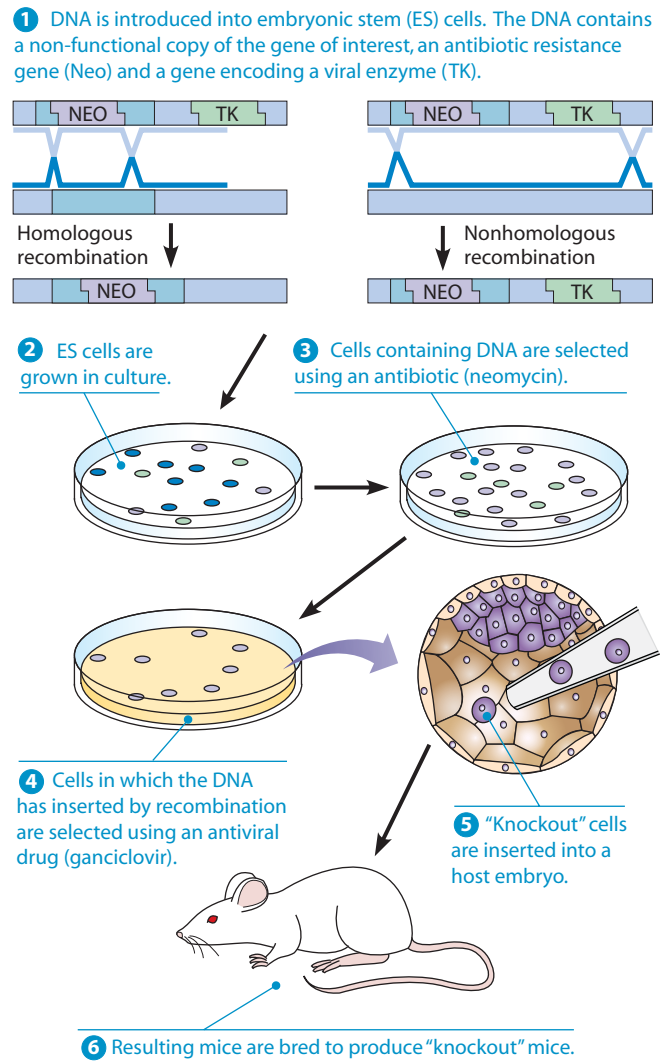


FIGURE 20-32 Making "Knockout" Mice by Homologous Recombination. The production of "knockout" mice involves ❶ the construction of a DNA targeting vector that contains a nonfunctional copy of the gene of interest, which ❷ is inserted into embryonic stem cells (ES cells). The ES cells are screened using two different drugs to select for rare cells in which a recombination event has occurred (❸, ❹). These "knockout" cells are ❺ inserted into a host embryo, and ❻ subsequent breeding produces mice carrying the engineered mutation.

BOX 20A TOOLS OF DISCOVERY

Making "Designer" Mice

A DNA fragment containing the gene of rat growth hormone was microinjected into the pronuclei of fertilized mouse eggs. Of 21 mice that developed from these eggs, seven carried the gene and six of these grew significantly larger than their littermates. (Palmiter et al., 1982, p. 611)

With these words, a team of investigators led by Richard Palmiter and Ralph Brinster reported how a genetic trait can be introduced experimentally into mice without going through the usual procedure of breeding—that is, sexual reproduction followed by the selection of desired traits. The researchers injected rat growth hormone genes into fertilized mouse eggs, and from one of these eggs a "supermouse" developed weighing almost twice as much as its littermates (**Figure 20A-1**). The accomplishment was heralded as a significant breakthrough because it proved the feasibility of applying genetic engineering to animals, with all the scientific and practical consequences such engineering is likely to have.

Palmiter, Brinster, and their colleagues began by isolating the gene for growth hormone (GH) from a library of rat DNA, using techniques similar to those described in this chapter. The cloned GH gene, with its regulatory region deleted, was then fused to the regulatory portion of a mouse gene—the gene that codes for *metallothionein (MT)*. MT is a small, metal-binding protein that is normally present in most mouse tissues and appears to be involved in regulating the level of zinc in the animal. The advantage of fusing the *MT* gene to the *GH* gene was that the expression of the *MT* gene could then be specifically induced (turned on) by zinc.

About 600 copies of the engineered DNA fragment were microinjected into fertilized mouse eggs, in a volume of about 2 picoliters (0.000002 μL!). The DNA was injected into the male pronucleus, the haploid sperm nucleus that has not yet fused with the haploid egg nucleus (**Figure 20A-2**). From the 170 fertilized eggs that were injected and implanted back into the reproductive tracts of foster mothers, 21 animals developed. Seven of them turned out to be transgenic mice with *MT–GH* genes present in their cells. In at least one case, a transgenic mouse transmitted the *MT–GH* gene faithfully to about half of its offspring, suggesting that the gene had become stably integrated into one of its chromosomes.

Because the *GH* gene had been linked to an *MT* gene regulatory region, it was predicted that the hybrid gene could be turned on by giving the mice zinc in their drinking water. The most dramatic evidence for expression of the rat *GH* genes was that the transgenic mice grew faster and weighed about twice as much as normal mice. During the period of maximum sensitivity to growth hormone (3 weeks to 3 months of age), the transgenic animals grew three to four times as fast as their normal littermates.

This dramatic experiment proved that it is possible to introduce cloned genes into the cells of higher organisms and that such genes can become stably integrated into the genome, where they are expressed and passed on to offspring. In the years since supermouse's creation, rapid progress has been made in most of these areas. Supermouse, it seems, was just the beginning.

FIGURE 20A-1 Genetic Engineering in Mice. "Supermouse" (on the left) is significantly larger than its littermate because it was engineered to carry, and express at high levels, the gene for rat growth hormone.

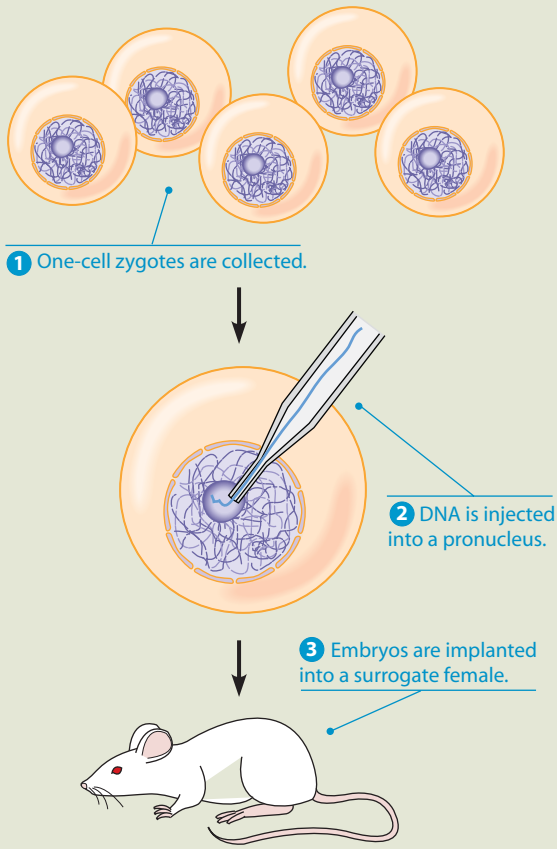


FIGURE 20A-2 Making Transgenic Mice. Fertilized mouse eggs (one-cell zygotes) are injected with DNA, typically into a pronucleus (either the nucleus derived from the sperm or egg). The injected zygotes are transferred into a surrogate mother during subsequent gestation.

VIDEOS www.thecellplace.com *Pronuclear injection*

human conditions, including cancer, obesity, heart disease, diabetes, arthritis, and aging.

Knockout technology has now been extended to other mammals besides mice. One use of this technology relates to *xenotransplantation,* the transplantation of tissues from one species to another. Pigs have been a major focus of such research, which aims to provide a source of temporary organs for human patients awaiting transplants. To avoid immune rejection, pigs have been produced that lack a key enzyme called *α-1,3-galactosyltransferase,* which normally catalyzes the addition of sugar residues to cell surfaces in pigs that contributes to the immune response.

Gene Therapies Are Being Developed for the Treatment of Human Diseases

Humans suffer from many diseases that might conceivably be cured by transplanting normal, functional copies of genes into people who possess defective, disease-causing genes. The success of transgenic and knockout mice, along with similar experiments, raised the question of whether gene transplantation techniques might eventually be applied to the problem of repairing defective genes in humans. Obvious candidates for such an approach, called *gene therapy,* include the inherited genetic diseases cystic fibrosis, hemophilia, hypercholesterolemia, hemoglobin disorders, muscular dystrophy, lysosomal storage diseases, and an immune disorder called *severe combined immunodeficiency (SCID).*

The first person to be treated using gene therapy was a 4-year-old girl with a type of SCID caused by a defect in the gene coding for *adenosine deaminase (ADA).* Loss of ADA activity leads to an inability to produce sufficient numbers of immune cells called *T lymphocytes.* As a result, the girl suffered from frequent and potentially life-threatening infections. In 1990, she underwent a series of treatments in which a normal copy of the cloned ADA gene was inserted into a virus, the virus was used to infect T lymphocytes obtained from the girl's blood, and the lymphocytes were then injected back into her bloodstream. The result was a significant improvement in her immune function, although the effect diminished over time, and the treatment did not seem to help most SCID patients.

In the years since these pioneering studies, considerable progress has been made in developing better techniques for delivering cloned genes into target cells and getting the genes to function properly. In the year 2000, French scientists finally reported what seemed to be a successful treatment for children with SCID (in this case, an especially severe form of SCID caused by a defective receptor gene rather than a defective ADA gene). By using a virus that was more efficient at transferring cloned genes and by devising better conditions for culturing cells during the gene transfer process, these scientists were able to restore normal levels of immune function to the children they treated. In fact, the outcome was so dramatic that, for the first time, the treated children were able to leave the protective isolation "bubble" that had been used in the hospital to shield them from infections.

It was therefore a great disappointment when three of the ten children treated in the initial study developed leukemia a few years later. Examination of the leukemia cells revealed that the virus used to deliver the corrective gene sometimes inserts itself next to a normal gene that, when expressed abnormally, can cause cancer to arise. (In Chapter 24 we will describe exactly how such an event, called *insertional mutagenesis,* can initiate cancer development.) We should not, of course, lose sight of the fact that these studies also provided one of the first hopeful signs that gene therapy can cure a life-threatening genetic disease. But the associated cancer risks must be better understood before such treatments will become practical.

One tactic for addressing the problem of cancer risk is to change the type of virus being used to ferry genes into target cells. The SCID studies employed *retroviruses* (page 648), which randomly insert themselves into chromosomal DNA and possess sequences that inadvertently activate adjacent host genes. Another type of virus being investigated as a vehicle for gene therapy, called *adeno-associated virus (AAV),* is less likely to insert directly into chromosomal DNA and less likely to inadvertently activate host genes when it does become inserted. Some encouraging results using this virus have been obtained in patients with *hemophilia,* an inherited disease characterized by life-threatening episodes of uncontrolled bleeding. Hemophilia is caused by genetic defects in proteins called *blood-clotting factors,* which participate in the formation of blood clots. In gene therapy trials, hemophilia patients have been injected with AAV containing a gene coding for the blood-clotting factor they require. When the patients' liver cells were infected, they produced enough blood-clotting factor to alleviate the uncontrolled bleeding normally associated with the disease. Although this "cure" lasted only about eight weeks because an immune response destroyed the modified liver cells, the immune reaction targeted a component of AAV that is only transiently present. It is therefore hoped that short-term administration of immunosuppressive drugs may help provide a more permanent cure.

In the years since the enormous potential of gene therapy was first publicized in the early 1980s, the field has been criticized for promising too much and delivering too little. But most new technologies take time to be perfected and encounter disappointments along the way, and gene therapy is no exception. Despite the setbacks, it appears likely that using normal genes to treat genetic diseases is a reachable goal that may one day become common practice, at least for a few genetic diseases that involve single gene defects. Of course, the ability to alter people's genes raises important ethical, safety, and legal concerns. The ultimate question of how society will control our growing power to change the human genome is an issue that will need to be thoroughly discussed not just by scientists and physicians, but by society as a whole.

SUMMARY OF KEY POINTS

Sexual Reproduction

- Asexual reproduction is based on mitotic cell division and produces offspring that are genetically identical (or nearly so) to the single parent. In contrast, sexual reproduction involves two parents and leads to a mixture of parental traits in the offspring.
- Sexual reproduction allows populations to adapt to environmental changes, enables desirable mutations to be combined in a single individual, and promotes genetic flexibility by maintaining a diploid genome.

Meiosis

- The life cycle of sexually reproducing eukaryotes includes both haploid and diploid phases. Haploid gametes are generated by meiosis and fuse at fertilization to restore the diploid chromosome number.
- Meiosis consists of two successive cell divisions without an intervening duplication of chromosomes. During the first meiotic division, homologous chromosomes separate and segregate into the two daughter cells. During the second meiotic division, sister chromatids separate and four haploid daughter cells are produced.
- In addition to reducing the chromosome number from diploid to haploid, meiosis differs from mitosis in that homologous chromosomes synapse during prophase of the first meiotic division, thereby allowing crossing over and genetic recombination between nonsister chromatids.

Genetic Variability: Segregation and Assortment of Alleles

- Mendel's laws of inheritance describe the genetic consequences of chromosome behavior during meiosis, even though chromosomes had not yet been discovered at the time of Mendel's experiments.
- Mendel's law of segregation states that the two (maternal and paternal) alleles of a gene are distinct entities that separate into different gametes during meiosis. The law of independent assortment states that the alleles of each gene separate independently of the alleles of other genes (now known to apply only to genes located on different chromosomes or very far apart on the same chromosome).

Genetic Variability: Recombination and Crossing Over

- The genetic variability among an organism's gametes arises partly from the independent assortment of chromosomes during anaphase I and partly from genetic recombination during prophase I.
- The frequency of recombination between genes on the same chromosome is a measure of the distance between the two genes and can therefore be used to map their chromosomal locations.

Genetic Recombination in Bacteria and Viruses

- Besides occurring during meiosis, homologous recombination also takes place in viruses (during co-infection) and when DNA is transferred into bacterial cells by transformation, transduction, or conjugation.

Molecular Mechanism of Homologous Recombination

- Recombination involves breakage and exchange between DNA molecules exhibiting extensive sequence homology.
- Recombination is sometimes accompanied by gene conversion or the formation of DNA molecules whose two strands are not completely complementary to one another. These phenomena can be explained by recombination models involving the formation of Holliday junctions, which are regions of single-strand exchange between double-stranded DNA molecules.

Recombinant DNA Technology and Gene Cloning

- Recombinant DNA technology makes it possible to combine DNA from any two (or more) sources into a single DNA molecule.
- Combining a gene of interest with a plasmid or phage cloning vector allows the gene to be cloned (amplified) in bacterial cells. This approach, as well as the polymerase chain reaction (PCR), permits large quantities of specific gene sequences and their protein products to be prepared for research and practical purposes.

Genetic Engineering

- Recombinant DNA technology has many practical applications in medicine and agriculture. These include the ability to produce valuable proteins that are otherwise difficult to obtain and the ability to improve the traits of food crops, such as enhanced resistance to insects, herbicides, or disease. Attempts are also underway to develop gene therapies for treating human diseases.

MAKING CONNECTIONS

In this chapter, you have learned how meiosis allows the genetic information in replicated DNA molecules to be recombined during the formation of sperm and egg cells, thereby making sexual reproduction possible. Thus, in contrast to mitotic cell division (described in Chapter 19), which creates cells that are virtually identical in their DNA makeup, meiosis creates cells containing new combinations of DNA sequences. Perhaps you now may be asking: How does DNA, which has been replicated and distributed to daughter cells during mitosis or meiosis, actually carry out its role as the source of the genetic information that specifies cellular structure and guides cellular behavior? In Chapter 21, we will begin to answer this question by exploring how DNA uses a genetic code to specify the amino acid sequence of protein molecules. The role of RNA molecules in this process will be covered in both Chapters 21 and 22. Finally, Chapter 23 examines the mechanisms used by cells for controlling expression of the genetic information stored in their DNA molecules.

PROBLEM SET

More challenging problems are marked with a •.

20-1 The Truth About Sex. For each of the following statements, indicate with an S if it is true of sexual reproduction, with an A if it is true of asexual reproduction, with a B if it is true of both, or with an N if it is true of neither.

(a) Traits from two different parents can be combined in a single offspring.

(b) Each generation of offspring is virtually identical to the previous generation.

(c) Mutations are propagated to the next generation.

(d) Some offspring in every generation will be less suited for survival than the parents, but others may be better suited.

(e) Mitosis is involved in the life cycle.

20-2 Ordering the Phases of Meiosis. Drawings of several phases of meiosis in an organism, labeled A through F, are shown in **Figure 20-33**.

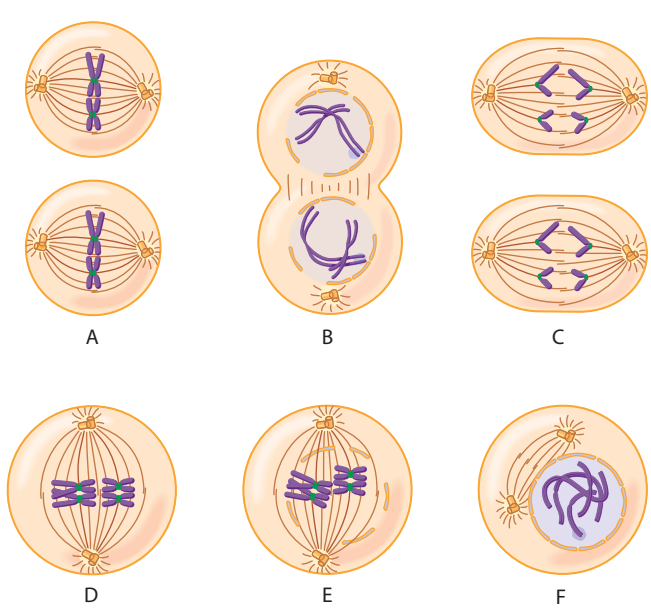


FIGURE 20-33 Six Phases of Meiosis to Be Ordered and Identified. See Problem 20-2.

(a) What is the diploid chromosome number in this species?

(b) Place the six phases in chronological order, and name each one.

(c) Between which two phases do homologous centromeres separate?

(d) Between which two phases does recombination occur?

20-3 Telling Them Apart. Briefly describe how you might distinguish between each of the following pairs of phases in the same organism:

(a) Metaphase of mitosis and metaphase I of meiosis.

(b) Metaphase of mitosis and metaphase II of meiosis.

(c) Metaphase I and metaphase II of meiosis.

(d) Telophase of mitosis and telophase II of meiosis.

(e) Pachytene and diplotene stages of meiotic prophase I.

20-4 Your Centromere Is Showing. Suppose you have a diploid organism in which all the chromosomes contributed by the sperm have cytological markers on their centromeres that allow you to distinguish them visually from the chromosomes contributed by the egg.

(a) Would you expect all the somatic cells (cells other than gametes) to have equal numbers of maternal and paternal centromeres in this organism? Explain.

(b) Would you expect equal numbers of maternal and paternal centromeres in each gamete produced by that individual? Explain.

20-5 How Much DNA? Let X be the amount of DNA present in the gamete of an organism that has a diploid chromosome number of 4. Assuming all chromosomes to be of approximately the same size, how much DNA (X, $2X$, $1/2X$, and so on) would you expect in each of the following?

(a) A zygote immediately after fertilization

(b) A single sister chromatid

(c) A daughter cell following mitosis

(d) A single chromosome following mitosis

(e) A nucleus in mitotic prophase

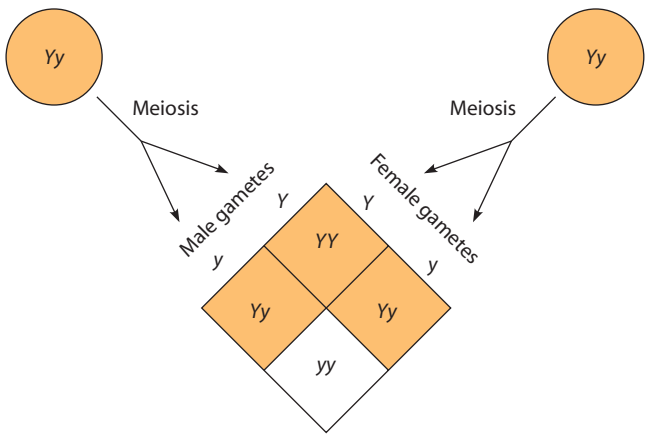
(f) The cell during metaphase II of meiosis

(g) One bivalent

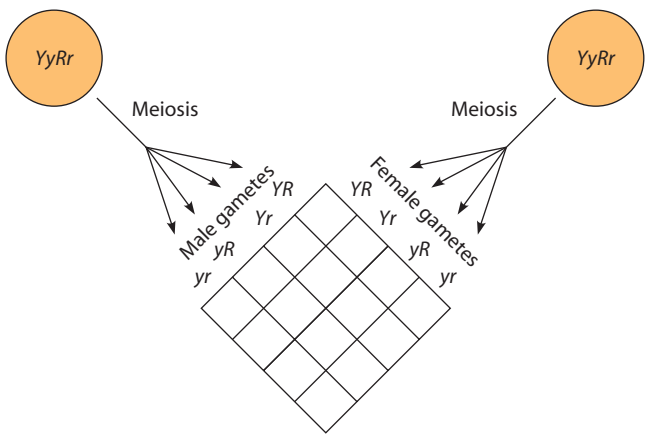
20-6 Meiotic Mistakes. Infants born with Patau syndrome have an extra copy of chromosome 13, which leads to developmental abnormalities such as cleft lip and palate, small eyes, and extra fingers and toes. Another type of genetic disorder, called Turner syndrome, results from the presence of only one sex chromosome—an X chromosome. Individuals born with one X chromosome are females exhibiting few noticeable defects until puberty, when they fail to develop normal breasts and internal sexual organs. Describe the meiotic events that could lead to the birth of an individual with either Patau syndrome or Turner syndrome.

20-7 Punnett Squares as Genetic Tools. A *Punnett square* is a diagram representing all possible outcomes of a genetic cross. The genotypes of all possible gametes from the male and female parents are arranged along two adjacent sides of a square, and each box in the matrix is then used to represent the genotype resulting from the union of the two gametes at the heads of the intersecting rows. By the law of independent assortment, all possible combinations are equally likely, so the frequency of a given genotype among the boxes represents the frequency of that genotype among the progeny of the genetic cross represented by the Punnett square.

Figure 20-34 shows the Punnett squares for two crosses of pea plants. The genetic characters involved are seed color



(a) One-factor cross



(b) Two-factor cross

FIGURE 20-34 Punnett Squares. See Problem 20-7.

(where *Y* is the allele for yellow seeds and *y* for green seeds) and seed shape (where *R* is the allele for round seeds and *r* for wrinkled seeds). The Punnett square in Figure 20-34a represents a one-factor cross between parent plants that are both heterozygous for seed color *(Yy × Yy)*. The Punnett square in Figure 20-34b is a two-factor cross between plants heterozygous for both seed color *(Yy)* and seed shape *(Rr)*.

(a) Using the Punnett square of Figure 20-34a, explain the 3:1 phenotypic ratio Mendel observed for the offspring of such a cross.

(b) Explain why the Punnett square of Figure 20-34b is a 4 × 4 matrix with 16 genotypes. In general, what is the mathematical relationship between the number of heterozygous allelic pairs being considered and the number of different kinds of gametes?

(c) How does the Punnett square of Figure 20-34b reflect Mendel's law of independent assortment?

(d) Complete the Punnett square of Figure 20-34b by writing in each of the possible progeny genotypes. How many different genotypes will be found in the progeny? In what ratios?

(e) For the case of Figure 20-34b, how many different phenotypes will be found in the progeny? In what ratios?

• **20-8 Genetic Mapping.** The following table provides data concerning the frequency with which four genes (*w, x, y,* and *z*) located on the same chromosome recombine with each other.

Genes	Recombination Frequency
w and *x*	25%
w and *y*	29%
w and *z*	17%
x and *y*	50%
x and *z*	9%
y and *z*	44%

(a) Construct a genetic map indicating the order in which these four genes occur and the number of map units that separate the genes from each other.

(b) In constructing this map, you may have noticed that the map distances are not exactly additive. Can you provide an explanation for this apparent discrepancy?

• **20-9 Homologous Recombination.** Bacterial cells use at least three different pathways for carrying out genetic recombination. All three pathways require the RecA protein, but in each case, a different set of steps precedes the action of RecA in catalyzing strand invasion. One of these three pathways utilizes an enzyme complex called RecBCD, which binds to double-strand breaks in DNA and exhibits both helicase and single-strand nuclease activities.

(a) Briefly describe a model showing how the RecBCD enzyme complex might set the stage for genetic recombination.

(b) When bacterial cells are co-infected with two different strains of bacteriophage 1, genes located near certain regions of the phage DNA, called *CHI sites,* recombine at much higher frequencies than other genes do. However, in mutant bacteria lacking the RecBCD protein, genes located near CHI sites do not recombine any more frequently than do other genes. How can you modify your model to accommodate this additional information?

20-10 Gene Cloning and Recombination. Not only are the plasmid vectors used in molecular biology engineered, but the strains of *E. coli* used in cloning are as well.

(a) Nearly all strains of *E. coli* used in DNA cloning carry mutations in the *recA* gene that result in loss of RecA activity. Why would a *recA* mutation make an *E. coli* cell a better host for propagating recombinant plasmid DNA?

(b) Recall that restriction endonucleases are normally made by bacteria such as *E. coli* (Box 18B, page 520). *E. coli* used in molecular biology also carry mutations in restriction endonucleases. Why do you think these mutations would be useful?

SUGGESTED READING

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