CHAPTER OUTLINE

Shell coiling in the water snail, Lymnaea peregra. *In this species, some snails coil to the left, and others coil to the right. This is due to an inheritance pattern called the maternal effect.*

5 NON-MENDELIAN INHERITANCE

Mendelian inheritance patterns involve genes that directly influence the outcome of an offspring's traits and obey Mendel's laws. To predict phenotype, we must consider several factors. These include the dominant/recessive relationship of alleles, gene interactions that may affect the expression of a single trait, and the roles that sex and the environment play in influencing the individual's phenotype. Once these factors are understood, we can predict the phenotypes of offspring from their genotypes.

Most genes in eukaryotic species follow a Mendelian pattern of inheritance. However, many genes do not. In this chapter, we will examine several additional and even bizarre types of inheritance patterns that deviate from a Mendelian pattern. In the first two sections, we will consider two interesting examples of non-Mendelian inheritance called the maternal effect and epigenetic inheritance. Even though these inheritance patterns involve genes on chromosomes within the cell nucleus, the genotype of the offspring does not directly govern their phenotype in ways predicted by Mendel. We will see how the timing of gene expression and gene inactivation can cause a non-Mendelian pattern of inheritance.

In the third section, we will examine deviations from Mendelian inheritance that arise because some genetic material is not located in the cell nucleus. Certain cellular organelles, such as mitochondria and chloroplasts, contain their own genetic material. We will survey the inheritance of organellar genes and a few other examples in which traits are influenced by genetic material that exists outside of the cell nucleus.

5.1 MATERNAL EFFECT

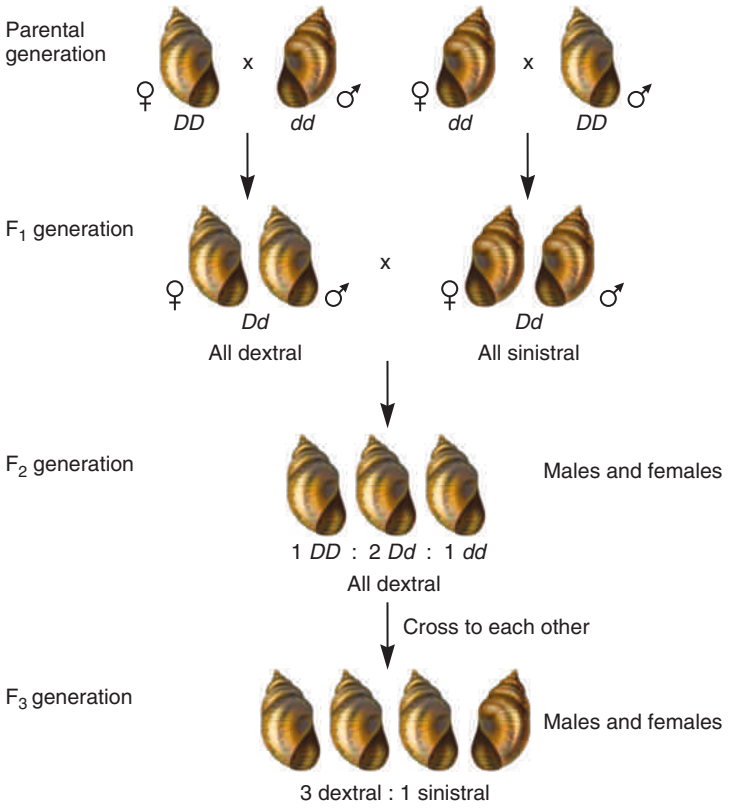
We will begin by considering genes that have a **maternal effect.** This term refers to an inheritance pattern for certain **nuclear genes**—genes located on chromosomes that are found in the cell nucleus—in which the genotype of the mother directly determines the phenotype of her offspring. Surprisingly, for maternal effect genes, the genotypes of the father and offspring themselves do not affect the phenotype of the offspring. We will see that this phenomenon is explained by the accumulation of gene products that the mother provides to her developing eggs.

The Genotype of the Mother Determines the Phenotype of the Offspring for Maternal Effect Genes

The first example of a maternal effect gene was studied in the 1920s by Arthur Boycott and involved morphological features of the water snail, *Lymnaea peregra*. In this species, the shell and

internal organs can be arranged in either a right-handed (dextral) or left-handed (sinistral) direction. The dextral orientation is more common and is dominant to the sinistral orientation. **Figure 5.1** describes the results of a genetic analysis carried out by Boycott. In this experiment, he began with two different true-breeding strains of snails with either a dextral or sinistral morphology. Many combinations of crosses produced results that could not be explained by a Mendelian pattern of inheritance. When a dextral female (*DD*) was crossed to a sinistral male (*dd*), all F_1 offspring were dextral. However, in the **reciprocal cross,** where a sinistral female (*dd*) was crossed to a dextral male (*DD*), all F_1 offspring were sinistral. Taken together, these results contradict a Mendelian pattern of inheritance.

How can we explain the unusual results obtained in Figure 5.1? Alfred Sturtevant proposed the idea that snail coiling is due to a maternal effect gene that exists as a dextral (*D*) or sinistral (*d*) allele. His conclusions were drawn from the inheritance patterns of the F_2 and F_3 generations. In this experiment, the genotype of the F_1 generation is expected to be heterozygous (*Dd*).



INTERACTIVE EXERCISE

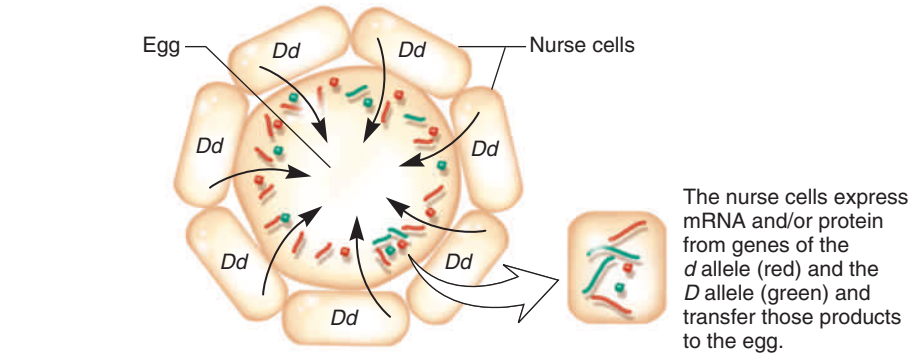
FIGURE 5.1 Experiment showing the inheritance pattern of snail coiling. In this experiment, *D* (dextral) is dominant to *d* (sinistral). The genotype of the mother determines the phenotype of the offspring. This phenomenon is known as the maternal effect. In this case, a *DD* or *Dd* mother produces dextral offspring, and a *dd* mother produces sinistral offspring. The genotypes of the father and offspring do not affect the offspring's phenotype.

When these F_1 individuals were crossed to each other, a genotypic ratio of 1 *DD* : 2 *Dd* : 1 *dd* is predicted for the F_2 generation. Because the *D* allele is dominant to the *d* allele, a 3:1 phenotypic ratio of dextral to sinistral snails should be produced according to a Mendelian pattern of inheritance. Instead of this predicted phenotypic ratio, however, the F_2 generation was composed of all dextral snails. This incongruity with Mendelian inheritance is due to the maternal effect. The phenotype of the offspring depended solely on the genotype of the mother. The F_1 mothers were *Dd*. The *D* allele in the mothers is dominant to the *d* allele and caused the offspring to be dextral, even if the offspring's genotype was *dd*. When the members of the F_2 generation were crossed, the F_3 generation exhibited a 3:1 ratio of dextral to sinistral snails. This ratio corresponds to the genotypes of the F_2 females, which were the mothers of the F_3 generation. The ratio of F_2 females was 1 *DD* : 2 *Dd* : 1 *dd*. The *DD* and *Dd* females produced dextral offspring, whereas the *dd* females produced sinistral offspring. This explains the 3:1 ratio of dextral and sinistral offspring in the F_3 generation.

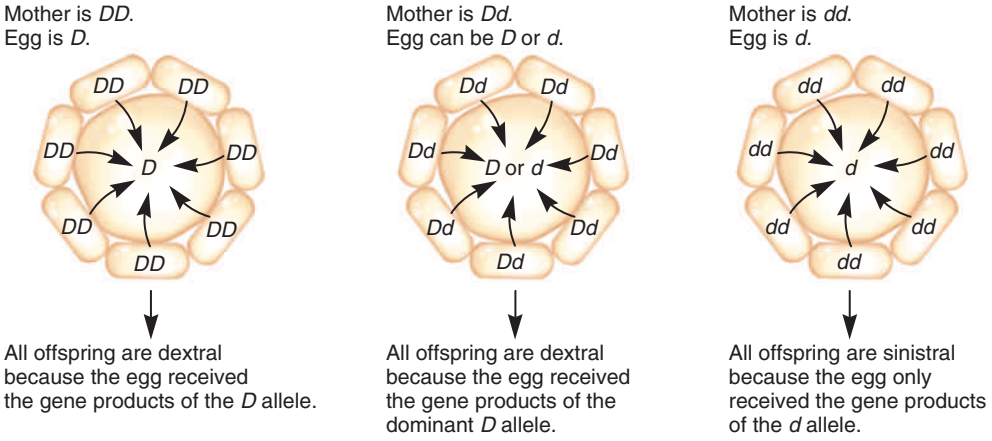
Female Gametes Receive Gene Products from the Mother That Affect Early Developmental Stages of the Embryo

At the molecular and cellular level, the non-Mendelian inheritance pattern of maternal effect genes can be explained by the process of oogenesis in female animals (**Figure 5.2a**). As an animal oocyte (egg) matures, many surrounding maternal cells called nurse cells provide the egg with nutrients and other materials. In Figure 5.2a, a female is heterozygous for the snail-coiling maternal effect gene, with the alleles designated *D* and *d*. Depending on the outcome of meiosis, the haploid egg may receive the *D* allele or the *d* allele, but not both. The surrounding nurse cells, however, produce both *D* and *d* gene products (mRNA and proteins). These gene products are then transported into the egg. As shown here, the egg has received both the *D* allele gene products and the *d* allele gene products. These gene products persist for a significant time after the egg has been fertilized and begins its embryonic development. In this way, the gene products of the nurse cells, which reflect the genotype of the mother, influence the early developmental stages of the embryo.

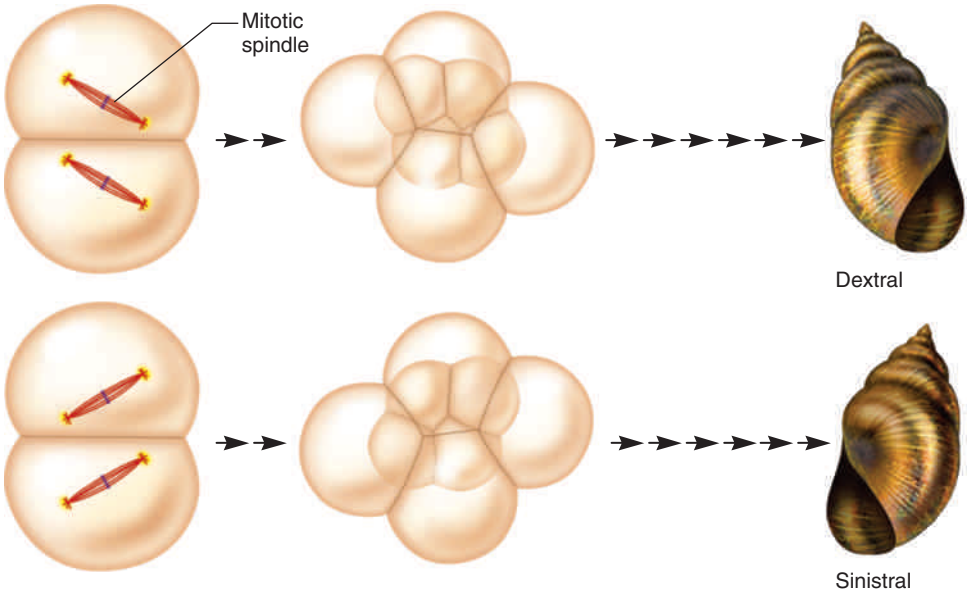
Now that we have an understanding of the relationship between oogenesis and maternal effect genes, let's reconsider the topic of snail coiling. As shown in **Figure 5.2b**, a female snail that is *DD* transmits only the *D* gene products to the egg. During the early stages of embryonic development, these gene products cause the egg cleavage to occur in a way that promotes a right-handed body plan. A heterozygous female transmits both *D* and *d* gene products. Because the *D* allele is dominant, the maternal effect also causes a right-handed body plan. Finally, a *dd* mother contributes only *d* gene products that promote a left-handed body plan, even if the egg is fertilized by a sperm carrying a *D* allele. The sperm's genotype is irrelevant, because the expression of the sperm's gene would occur too late. The origin of dextral and sinistral coiling can be traced to the orientation



(a) Transfer of gene products from nurse cells to egg



(b) Maternal effect in snail coiling



(c) An explanation of coiling direction at the cellular level

FIGURE 5.2 **The mechanism of maternal effect in snail coiling.** **(a)** Transfer of gene products from nurse cells to an egg. The nurse cells are heterozygous (*Dd*). Both the *D* and *d* alleles are activated in the nurse cells to produce *D* and *d* gene products (mRNA or proteins, or both). These products are transported into the cytoplasm of the egg, where they accumulate to significant amounts. **(b)** Explanation of the maternal effect in snail coiling. **(c)** The direction of snail coiling is determined by differences in the cleavage planes during early embryonic development.

Genes → Traits If the nurse cells are *DD* or *Dd*, they will transfer the *D* gene product to the egg and thereby cause the resulting offspring to be dextral. If the nurse cells are *dd*, only the *d* gene product will be transferred to the egg, so the resulting offspring will be sinistral.

of the mitotic spindle at the two- to four-cell stage of embryonic development. The dextral and sinistral snails develop as mirror images of each other (**Figure 5.2c**).

Since these initial studies, researchers have found that maternal effect genes encode proteins that are important in the early steps of embryogenesis. The accumulation of maternal gene products in the egg allows embryogenesis to proceed quickly after fertilization. Maternal effect genes often play a role in cell division, cleavage pattern, and body axis orientation. Therefore, defective alleles in maternal effect genes tend to have a dramatic effect on the phenotype of the individual, altering major features of morphology, often with dire consequences.

Our understanding of maternal effect genes has been greatly aided by their identification in experimental organisms such as *Drosophila melanogaster*. In such organisms with a short generation time, geneticists have successfully searched for mutant alleles that prevent the normal process of embryonic development. In *Drosophila*, geneticists have identified several maternal effect genes with profound effects on the early stages of development. The pattern of development of a *Drosophila* embryo occurs along axes, such as the anteroposterior axis and the dorsoventral axis. The proper development of each axis requires a distinct set of maternal gene products. For example, the maternal effect gene called *bicoid* produces a gene product that accumulates in a region of the egg that will eventually become anterior structures in the developing embryo. Mutant alleles of maternal effect genes often lead to abnormalities in the anteroposterior or the dorsoventral pattern of development. More recently, several maternal effect genes have been identified in mice and humans that are required for proper embryonic development. Chapter 23 examines the relationships among the actions of several maternal effect genes during embryonic development.

5.2 EPIGENETIC INHERITANCE

Epigenetic inheritance is a pattern in which a modification occurs to a nuclear gene or chromosome that alters gene expression, but is not permanent over the course of many generations. As we will see, epigenetic inheritance patterns are the result of DNA and chromosomal modifications that occur during oogenesis, spermatogenesis, or early stages of embryogenesis. Once they are initiated during these early stages, epigenetic changes alter the expression of particular genes in a way that may be fixed during an individual's lifetime. Therefore, epigenetic changes can permanently affect the phenotype of the individual. However, epigenetic modifications are not permanent over the course of many generations, and they do not change the actual DNA sequence. For example, a gene may undergo an epigenetic change that inactivates it for the lifetime of an individual. However, when this individual makes gametes, the gene may become activated and remain operative during the lifetime of an offspring who inherits the active gene.

In this section, we will examine two examples of epigenetic inheritance called dosage compensation and genomic imprinting. Dosage compensation has the effect of offseting differences in the number of sex chromosomes. One of the sex chromosomes is altered, with the result that males and females have similar levels of gene expression even though they do not possess the same complement of sex chromosomes. In mammals, dosage compensation is initiated during the early stages of embryonic development. By comparison, genomic imprinting happens prior to fertilization; it involves a change in a single gene or chromosome during gamete formation. Depending on whether the modification occurs during spermatogenesis or oogenesis, imprinting governs whether an offspring expresses a gene that has been inherited from its mother or father.

Dosage Compensation Is Necessary to Ensure Genetic Equality Between the Sexes

Dosage compensation refers to the phenomenon that the level of expression of many genes on the sex chromosomes (such as the X chromosome) is similar in both sexes even though males and females have a different complement of sex chromosomes. This term was coined in 1932 by Hermann Muller to explain the effects of eye color mutations in *Drosophila*. Muller observed that female flies homozygous for certain X-linked eye color alleles had a similar phenotype to hemizygous males. He noted that an X-linked gene conferring an apricot eye color produces a very similar phenotype in homozygous females and hemizygous males. In contrast, a female that has one copy of the apricot allele and a deletion of the apricot gene on the other X chromosome has eyes of paler color. Therefore, one copy of the allele in the female is not equivalent to one copy of the allele in the male. Instead, two copies of the allele in the female produce a phenotype that is similar to that produced by one copy in the male. In other words, the difference in gene dosage—two copies in females versus one copy in males—is being compensated for at the level of gene expression.

Since these initial studies, dosage compensation has been studied extensively in mammals, *Drosophila*, and *Caenorhabditis elegans* (a nematode). Depending on the species, dosage compensation occurs via different mechanisms (**Table 5.1**). Female mammals equalize the expression of X-linked genes by turning off one of their two X chromosomes. This process is known as **X inactivation.** In *Drosophila*, the male accomplishes dosage compensation by doubling the expression of most X-linked genes. In *C. elegans*, the XX animal is a hermaphrodite that produces both sperm and egg cells, and an animal carrying a single X chromosome is a male that produces only sperm. The XX hermaphrodite diminishes the expression of X-linked genes on both X chromosomes to approximately 50% of that in the male.

In birds, the Z chromosome is a large chromosome, usually the fourth or fifth largest, which contains almost all of the known sex-linked genes. The W chromosome is generally a much smaller microchromosome containing a high proportion of repeat sequence DNA that does not encode genes. Males are ZZ and females are ZW. Several years ago, researchers studied the level of expression of a Z-linked gene that encodes an enzyme called aconitase. They discovered that males express twice as much aconitase as females do. These results suggested

TABLE 5.1
Mechanisms of Dosage Compensation Among Different Species

Species	Sex Chromosomes in: Females	Males	Mechanism of Compensation
Placental mammals	XX	XY	One of the X chromosomes in the somatic cells of females is inactivated. In certain species, the paternal X chromosome is inactivated, and in other species, such as humans, either the maternal or paternal X chromosome is randomly inactivated throughout the somatic cells of females.
Marsupial mammals	XX	XY	The paternally derived X chromosome is inactivated in the somatic cells of females.
Drosophila melanogaster	XX	XY	The level of expression of genes on the X chromosome in males is increased twofold.
Caenorhabditis elegans	XX*	X0	The level of expression of genes on both X chromosomes in hermaphrodites is decreased to 50% levels compared with males.

*In *C. elegans*, an XX individual is a hermaphrodite, not a female.

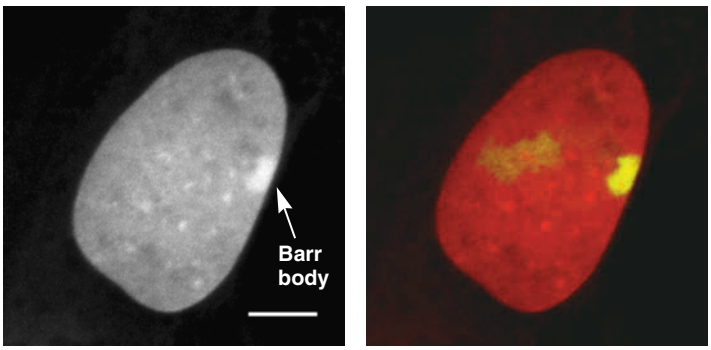
that dosage compensation does not occur in birds. More recently, the expression of hundreds of Z-linked genes has been examined in chickens. These newer results also suggest that birds lack a general mechanism of dosage compensation that controls the expression of most Z-linked genes. Even so, the pattern of gene expression between males and females was found to vary a great deal for certain Z-linked genes. Overall, the results suggest that some Z-linked genes may be dosage-compensated, but many of them are not.

Dosage Compensation Occurs in Female Mammals by the Inactivation of One X Chromosome

In 1961, Mary Lyon proposed that dosage compensation in mammals occurs by the inactivation of a single X chromosome in females. Liane Russell also proposed the same idea around the same time. This proposal brought together two lines of study. The first type of evidence came from cytological studies. In 1949, Murray Barr and Ewart Bertram identified a highly condensed structure in the interphase nuclei of somatic cells in female cats that was not found in male cats. This structure became known as the **Barr body** (Figure 5.3a). In 1960, Susumu Ohno correctly proposed that the Barr body is a highly condensed X chromosome.

In addition to this cytological evidence, Lyon was also familiar with mammalian examples in which the coat color had a variegated pattern. Figure 5.3b is a photo of a calico cat, which is a female that is heterozygous for an X-linked gene that can occur as an orange or a black allele. (The white underside is due to a dominant allele in a different gene.) The orange and black patches are randomly distributed in different female individuals. The calico pattern does not occur in male cats, but similar kinds of mosaic patterns have been identified in the female mouse. Lyon suggested that both the Barr body and the calico pattern are the result of X inactivation in the cells of female mammals.

The mechanism of X inactivation, also known as the **Lyon hypothesis,** is schematically illustrated in Figure 5.4. This example involves a white and black variegated coat color found in certain strains of mice. As shown here, a female mouse has inherited an X chromosome from its mother that carries an allele conferring white coat color (X^b). The X chromosome from its father carries a black coat color allele (X^B). How can X inactivation explain a variegated coat pattern? Initially, both X chromosomes are active. However, at an early stage of embryonic development, one of the two X chromosomes is randomly inactivated in each somatic cell and becomes a Barr body. For example, one embryonic cell may have the X^B chromosome inactivated. As the embryo continues



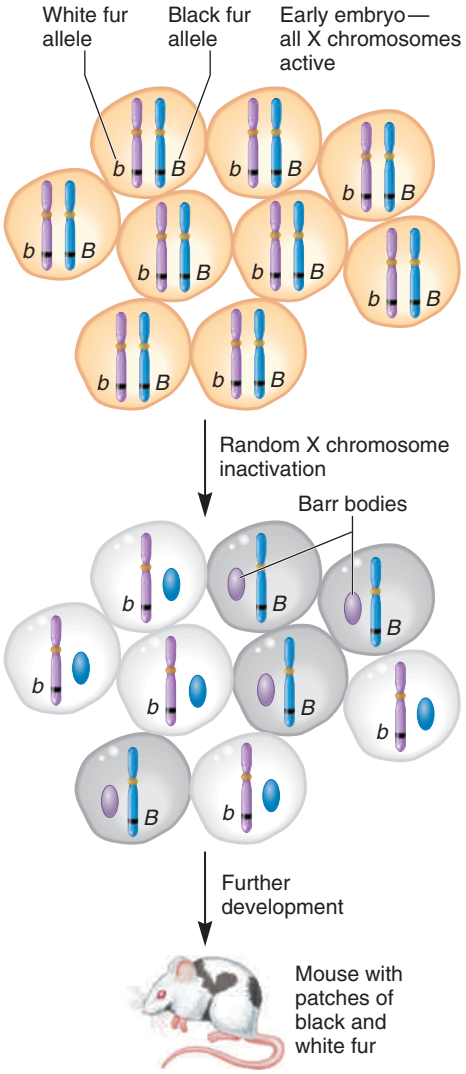
(a) Nucleus with a Barr body

(b) A calico cat

INTERACTIVE EXERCISE

FIGURE 5.3 **X chromosome inactivation in female mammals.** **(a)** The left micrograph shows the Barr body on the periphery of a human nucleus after staining with a DNA-specific dye. Because it is compact, the Barr body is the most brightly staining. The white scale bar is 5 μm. The right micrograph shows the same nucleus using a yellow fluorescent probe that recognizes the X chromosome. The Barr body is more compact than the active X chromosome, which is to the left of the Barr body. **(b)** The fur pattern of a calico cat.

Genes → Traits The pattern of black and orange fur on this cat is due to random X inactivation during embryonic development. The orange patches of fur are due to the inactivation of the X chromosome that carries a black allele; the black patches are due to the inactivation of the X chromosome that carries the orange allele. In general, only heterozygous female cats can be calico. A rare exception would be a male cat (XXY) that has an abnormal composition of sex chromosomes.



to grow and mature, this embryonic cell will divide and may eventually give rise to billions of cells in the adult animal. The epithelial (skin) cells that are derived from this embryonic cell will produce a patch of white fur because the X^B chromosome has been permanently inactivated. Alternatively, another embryonic cell may have the other X chromosome inactivated (i.e., X^b). The epithelial cells derived from this embryonic cell will produce a patch of black fur. Because the primary event of X inactivation is a random process that occurs at an early stage of development, the result is an animal with some patches of white fur and other patches of black fur. This is the basis of the variegated phenotype.

During inactivation, the chromosomal DNA becomes highly compacted in a Barr body, so most of the genes on the inactivated X chromosome cannot be expressed. When cell division occurs and the inactivated X chromosome is replicated, both copies remain highly compacted and inactive. Likewise, during subsequent cell divisions, X inactivation is passed along to all future somatic cells.

FIGURE 5.4 The mechanism of X chromosome inactivation.

Genes → Traits The top of this figure represents a mass of several cells that compose the early embryo. Initially, both X chromosomes are active. At an early stage of embryonic development, random inactivation of one X chromosome occurs in each cell. This inactivation pattern is maintained as the embryo matures into an adult.

EXPERIMENT 5A

In Adult Female Mammals, One X Chromosome Has Been Permanently Inactivated

According to the Lyon hypothesis, each somatic cell of female mammals expresses the genes on one of the X chromosomes, but not both. If an adult female is heterozygous for an X-linked gene, only one of two alleles will be expressed in any given cell. In 1963, Ronald Davidson, Harold Nitowsky, and Barton Childs set out to test the Lyon hypothesis at the cellular level. To do so, they analyzed the expression of a human X-linked gene that encodes an enzyme involved with sugar metabolism known as glucose-6-phosphate dehydrogenase (G-6-PD).

Prior to the Lyon hypothesis, biochemists had found that individuals vary with regard to the G-6-PD enzyme. This variation can be detected when the enzyme is subjected to gel electrophoresis (see the Appendix for a description of gel electrophoresis). One *G-6-PD* allele encodes a G-6-PD enzyme that migrates very quickly during gel electrophoresis (the "fast" enzyme), whereas another *G-6-PD* allele produces an enzyme that migrates more slowly (the "slow" enzyme). As shown in **Figure 5.5**, a sample of cells from heterozygous adult females produces both types of enzymes, whereas hemizygous males produce either the fast or slow type. The difference in migration between the fast and slow G-6-PD enzymes is due to minor differences in the structures of these enzymes. These minor differences do not significantly affect G-6-PD function, but they do enable geneticists to distinguish the proteins encoded by the two X-linked alleles.

As shown in **Figure 5.6**, Davidson, Nitowsky, and Childs tested the Lyon hypothesis using cell culturing techniques. They removed small samples of epithelial cells from a heterozygous female and grew them in the laboratory. When combined, these samples contained a mixture of both types of enzymes because

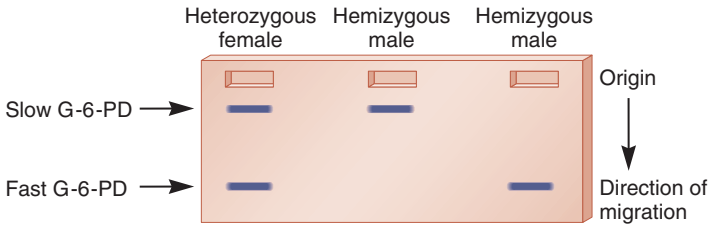


FIGURE 5.5 **Mobility of G-6-PD protein on gels.** *G-6-PD* can exist as a fast allele that encodes a protein that migrates more quickly to the bottom of the gel and a slow allele that migrates more slowly. The protein encoded by the fast allele is closer to the bottom of the gel.

the adult cells were derived from many different embryonic cells, some that had the slow allele inactivated and some that had the fast allele inactivated. In the experiment of Figure 5.6, these cells were sparsely plated onto solid growth media. After several days, each cell grew and divided to produce a colony, also called a **clone** of cells. All cells within a colony were derived from a single cell. The researchers reasoned that all cells within a single clone would express only one of the two *G-6-PD* alleles if the Lyon hypothesis was correct. Nine colonies were grown in liquid cultures, and then the cells were lysed to release the G-6-PD proteins inside of them. The proteins were then subjected to sodium dodecyl sulfate (SDS) gel electrophoresis.

THE HYPOTHESIS

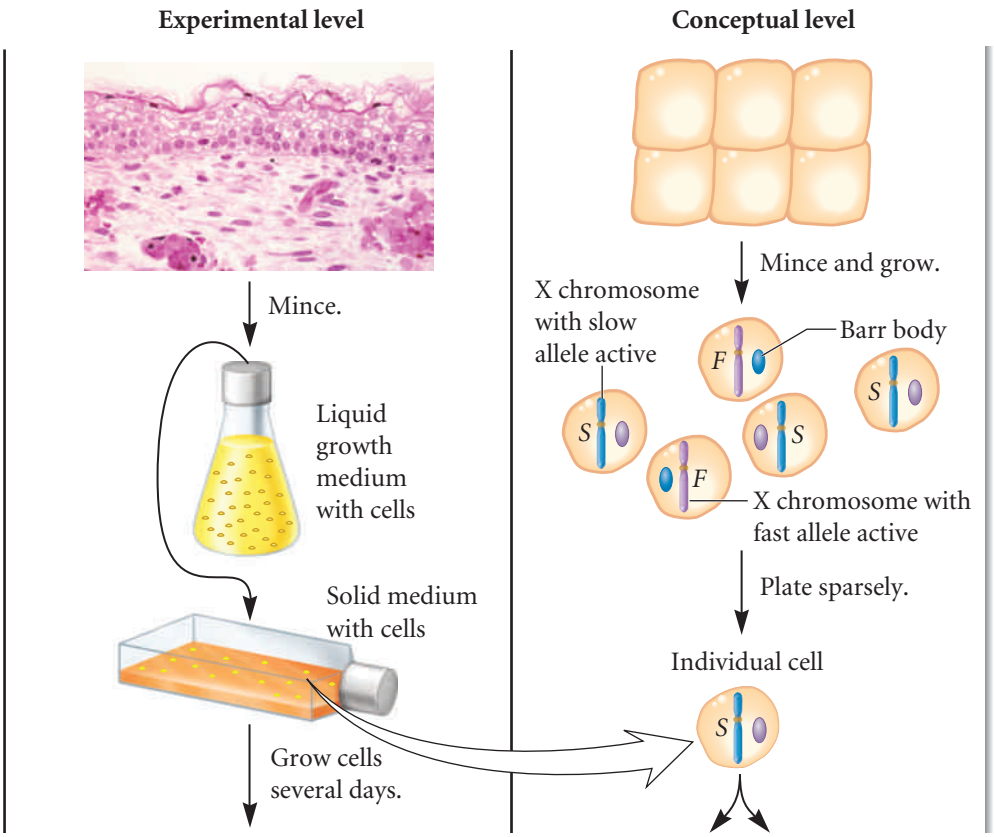
According to the Lyon hypothesis, an adult female who is heterozygous for the fast and slow *G-6-PD* alleles should express only one of the two alleles in any particular somatic cell and its descendants, but not both.

TESTING THE HYPOTHESIS — FIGURE 5.6 Evidence that adult female mammals contain one X chromosome that has been permanently inactivated.

Starting material: Small skin samples taken from a woman who was heterozygous for the fast and slow alleles of *G-6-PD*.

1. Mince the tissue to separate the individual cells.

2. Grow the cells in a liquid growth medium and then plate (sparsely) onto solid growth medium. The cells then divide to form a clone of many cells.

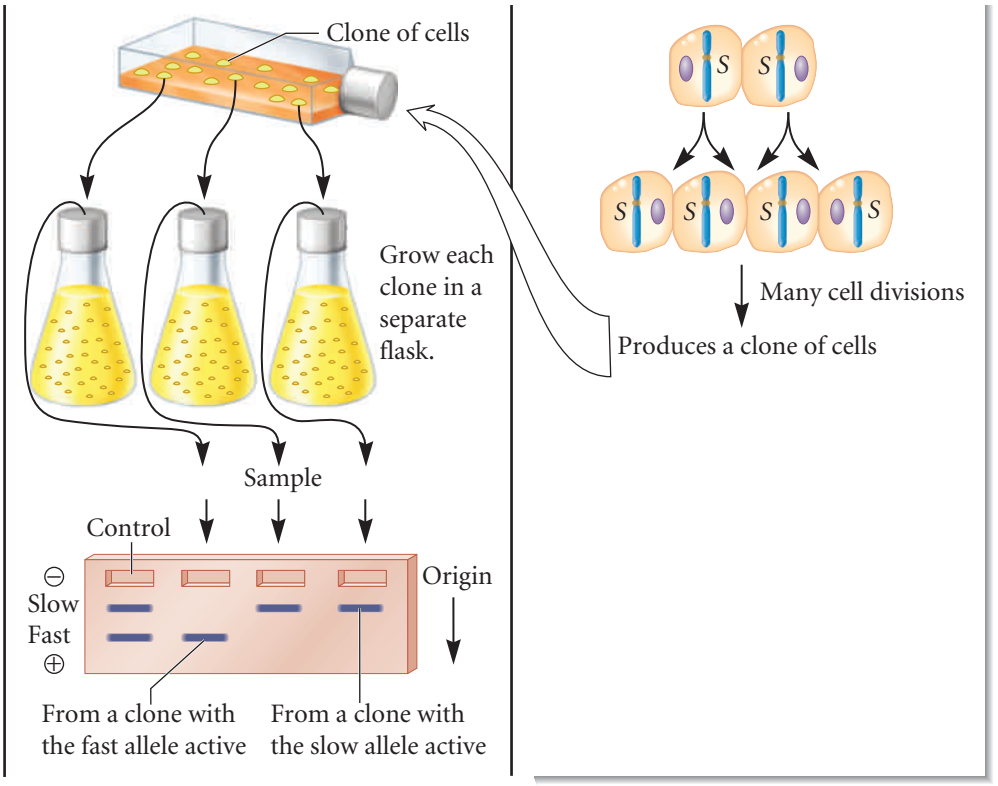


(continued)

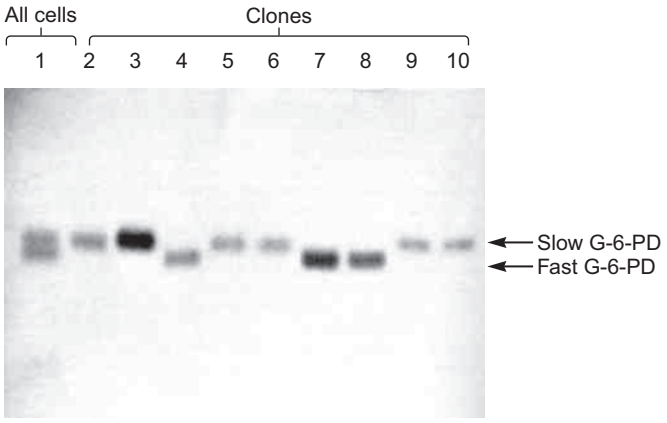
3. Take nine isolated clones and grow in liquid cultures. (Only three are shown here.)

4. Take cells from the liquid cultures, lyse cells to obtain proteins, and subject to gel electrophoresis. (This technique is described in the Appendix.)

   Note: As a control, lyse cells from step 1, and subject the proteins to gel electrophoresis. This control sample is not from a clone. It is a mixture of cells derived from a woman's skin sample.



THE DATA



Data from Ronald G. Davidson, Harold M. Nitowsky, and Barton Childs (1963) Demonstration of two population of cells in the human female heterozygous for glucose-6-phosphate dehydrogenase variants. *Proc Natl Acad Sci USA 50*, 481-485.

INTERPRETING THE DATA

In the data shown in Figure 5.6, the control (lane 1) was a protein sample obtained from a mixture of epithelial cells from a heterozygous woman who produced both types of G-6-PD enzymes. Bands corresponding to the fast and slow enzymes were observed in this lane. As described in steps 2 to 4, this mixture of epithelial cells was also used to generate nine clones. The proteins obtained from these clones are shown in lanes 2 through 10. Each clone was a population of cells independently derived from a single epithelial cell. Because the epithelial cells were obtained from an adult female, the Lyon hypothesis predicts that each epithelial cell would already have one of its X chromosomes permanently inactivated and would pass this trait to its progeny cells. For example, suppose that an epithelial cell had inactivated the X chromosome that encoded the fast G-6-PD. If this cell was allowed to form a clone of cells on a plate, all cells in this clonal population would be expected to have the same X chromosome inactivated—the X chromosome encoding the fast G-6-PD. Therefore, this clone of cells should express only the slow G-6-PD. As shown in the data, all nine clones expressed either the fast or slow G-6-PD protein, but not both. These results are consistent with the hypothesis that X inactivation has already occurred in any given epithelial cell and that this pattern of inactivation is passed to all of its progeny cells.

A self-help quiz involving this experiment can be found at www.mhhe.com/brookergenetics4e.

Mammals Maintain One Active X Chromosome in Their Somatic Cells

Since the Lyon hypothesis was confirmed, the genetic control of X inactivation has been investigated further by several laboratories. Research has shown that mammalian cells possess the ability to count their X chromosomes in their somatic cells and allow only one of them to remain active. How was this determined? A key observation came from comparisons of the chromosome composition of people who were born with normal or abnormal numbers of sex chromosomes.

Phenotype	Chromosome Composition	Number of X Chromosomes	Number of Barr Bodies
Normal female	XX	2	1
Normal male	XY	1	0
Turner syndrome (female)	X0	1	0
Triple X syndrome (female)	XXX	3	2
Klinefelter syndrome (male)	XXY	2	1

In normal females, two X chromosomes are counted and one is inactivated, while in males, one X chromosome is counted and none inactivated. If the number of X chromosomes exceeds two, as in triple X syndrome, additional X chromosomes are converted to Barr bodies.

X Inactivation in Mammals Depends on the X-Inactivation Center and the *Xist* Gene

Although the genetic control of inactivation is not entirely understood at the molecular level, a short region on the X chromosome called the **X-inactivation center (Xic)** is known to play a critical role (**Figure 5.7**). Eeva Therman and Klaus Patau identified the Xic from its key role in X inactivation. The counting of human X chromosomes is accomplished by counting the number of Xics. A Xic must be found on an X chromosome for inactivation to occur. Therman and Patau discovered that if one of the two X chromosomes in a female is missing its Xic due to a chromosome mutation, a cell counts only one Xic and X inactivation does not occur. Having two active X chromosomes is a lethal condition for a human female embryo.

Let's consider how the molecular expression of certain genes controls X inactivation. The expression of a specific gene within the Xic is required for the compaction of the X chromosome into a Barr body. This gene, discovered in 1991, is named *Xist* (for X-inactive specific transcript). The *Xist* gene on the inactivated X chromosome is active, which is unusual because most other genes

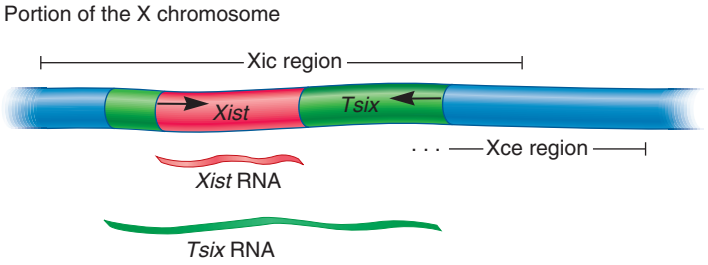


FIGURE 5.7 The X-inactivation center (Xic) of the X chromosome. The *Xist* gene is transcribed into RNA from the inactive X chromosome but not from the active X chromosome. This *Xist* RNA binds to the inactive X chromosome and recruits proteins that promote its compaction. The precise location of the Xce is not known. Some of it is adjacent to Xic and some of it may include all or part of the Xic. Xce may regulate the transcription of the *Xist* or *Tsix* genes and thereby influence the choice of the X chromosome that remains active.

on the inactivated X chromosome are silenced. The *Xist* gene product is an RNA molecule that does not encode a protein. Instead, the role of the *Xist* RNA is to coat the X chromosome and inactivate it. After coating, other proteins associate with the *Xist* RNA and promote chromosomal compaction into a Barr body.

A second gene found within the Xic, designated *Tsix*, plays a role in preventing X inactivation. As shown in Figure 5.7, the *Xist* and *Tsix* genes are overlapping and transcribed in opposite directions. (The name *Tsix* is *Xist* spelled backwards). The expression of the *Tsix* gene inhibits the transcription of the *Xist* gene. On the active X chromosome, the *Tsix* gene is expressed, and the *Xist* gene is not. The opposite situation occurs on the inactive X chromosome—*Xist* is expressed, and *Tsix* is not. Researchers have studied heterozygous females that carry a normal *Tsix* gene on one X chromosome and a defective, mutant *Tsix* gene on the other. The X chromosome carrying the mutant *Tsix* gene is preferentially inactivated.

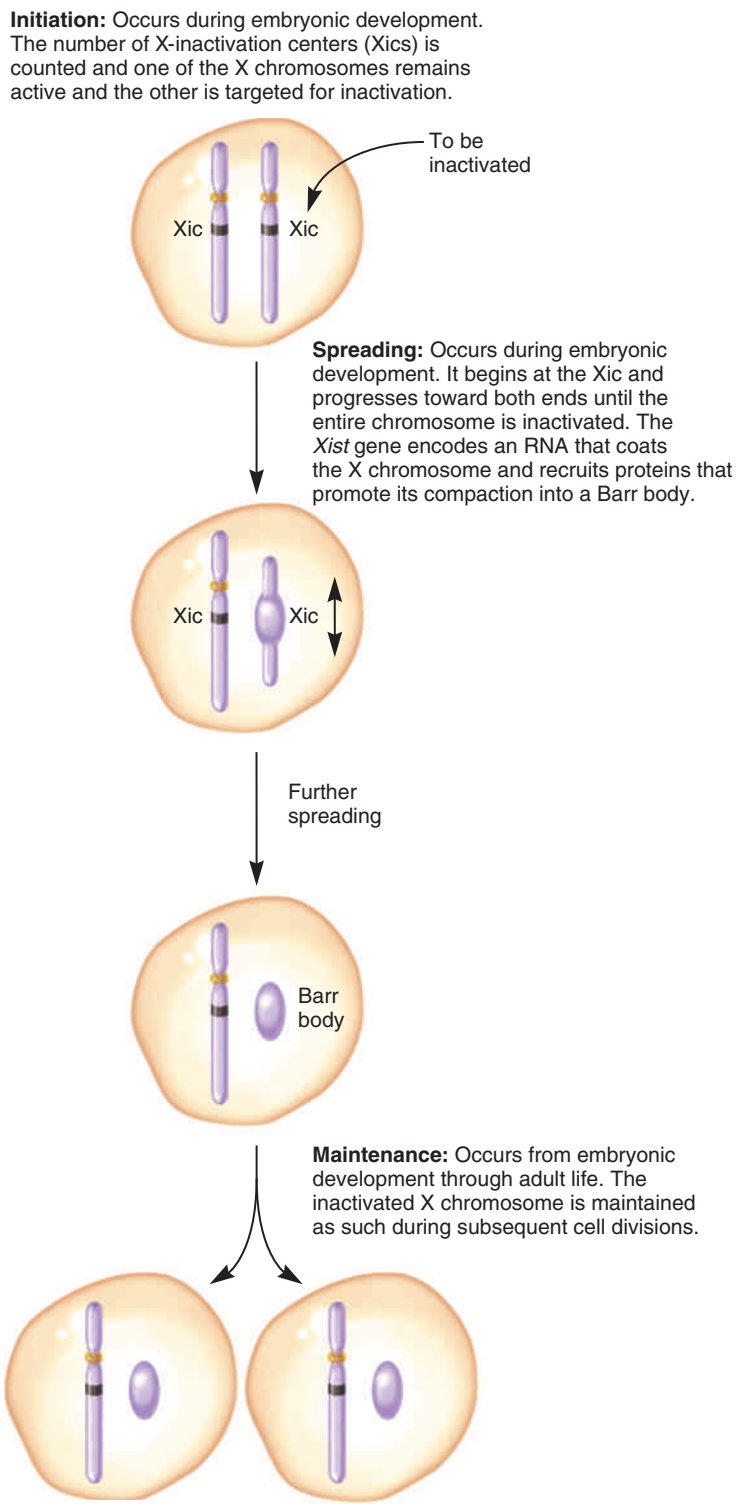
Another region termed the **X chromosomal controlling element (Xce)** also affects the choice of the X chromosome to be inactivated. Genetic variation occurs in the Xce. An X chromosome that carries a strong Xce is more likely to remain active than an X chromosome that carries a weak Xce, thereby leading to skewed (nonrandom) X inactivation. As shown in Figure 5.7, the Xce is very close to the end of the Xic and may even encompass all or part of the Xic. Although the mechanism by which the Xce exerts its effects are not well understood, some researchers speculate that Xce serves as a binding site for proteins that regulate the expression of genes in the Xic, such as *Xist* or *Tsix*. Genetic variation in Xce that enhances *Xist* expression would tend to promote Barr body formation, whereas Xce variation that enhances *Tsix* expression would tend to prevent X inactivation.

X Inactivation Occurs in Three Phases: Initiation, Spreading, and Maintenance

The process of X inactivation can be divided into three phases: initiation, spreading, and maintenance (**Figure 5.8**). During initiation, which occurs during embryonic development, one of the X chromosomes remains active, and the other is chosen to be inactivated. How is a particular X chromosome chosen for X inactivation? The answer is not well understood, but is thought to involve a complex interplay between *Xist* and *Tsix* gene expression.

During the spreading phase, the chosen X chromosome is inactivated. This spreading requires the expression of the *Xist* gene. The *Xist* RNA coats the inactivated X chromosome and recruits proteins that promote compaction. This compaction involves DNA methylation and also the modification of histone proteins, which are described in Chapter 10. The spreading phase is so named because inactivation begins near the Xic and spreads in both directions along the X chromosome.

Once the initiation and spreading phases occur for a given X chromosome, the inactivated X chromosome is maintained as a Barr body during future cell divisions. When a cell divides, the Barr body is replicated, and both copies remain compacted. This maintenance phase continues from the embryonic stage through adulthood.



ONLINE ANIMATION

FIGURE 5.8 The function of the Xic during X chromosome inactivation.

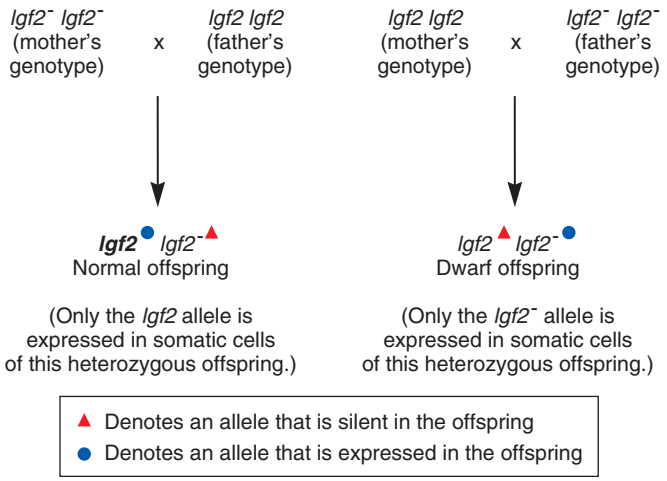
Some genes on the inactivated X chromosome are expressed in the somatic cells of adult female mammals. These genes are said to escape the effects of X inactivation. As mentioned, *Xist* is an example of a gene that is expressed from the highly condensed Barr body. In humans, up to a quarter of X-linked genes may escape inactivation to some degree. Many of these genes occur in clusters. Among these are the pseudoautosomal genes found on the X and Y chromosomes in the regions of homology described in Chapter 4. Dosage compensation is not necessary for X-linked pseudoautosomal genes because they are located on both the X and Y chromosomes. How are genes on the Barr body expressed? Although the mechanism is not understood, these genes may be found in localized regions where the chromatin is less tightly packed and able to be transcribed.

The Expression of an Imprinted Gene Depends on the Sex of the Parent from Which the Gene Was Inherited

As we have just seen, dosage compensation changes the level of expression of many genes located on the X chromosome. We now turn to another epigenetic phenomenon known as imprinting. The term imprinting implies a type of marking process that has a memory. For example, newly hatched birds identify marks on their parents, which allows them to distinguish their parents from other individuals. The term **genomic imprinting** refers to an analogous situation in which a segment of DNA is marked, and that mark is retained and recognized throughout the life of the organism inheriting the marked DNA. The phenotypes caused by imprinted genes follow a non-Mendelian pattern of inheritance because the marking process causes the offspring to distinguish between maternally and paternally inherited alleles. Depending on how the genes are marked, the offspring expresses only one of the two alleles. This phenomenon is termed **monoallelic expression.**

To understand genomic imprinting, let's consider a specific example. In the mouse, a gene designated *Igf2* encodes a protein growth hormone called insulin-like growth factor 2. Imprinting occurs in a way that results in the expression of the paternal *Igf2* allele but not the maternal allele. The paternal allele is transcribed into RNA, but the maternal allele is transcriptionally silent. With regard to phenotype, a functional *Igf2* gene is necessary for normal size. A loss-of-function allele of this gene, designated $Igf2^{-}$, is defective in the synthesis of a functional Igf2 protein. This may cause a mouse to be a dwarf, but the dwarfism depends on whether the mutant allele is inherited from the male or female parent, as shown in **Figure 5.9**. On the left side, an offspring has inherited the *Igf2* allele from its father and the $Igf2^{-}$ allele from its mother. Due to imprinting, only the *Igf2* allele is expressed in the offspring. Therefore, this mouse grows to a normal size. Alternatively, in the reciprocal cross on the right side, an individual has inherited the $Igf2^{-}$ allele from its father and the *Igf2* allele from its mother. In this case, the *Igf2* allele is not expressed. In this mouse, the $Igf2^{-}$ allele would be transcribed into mRNA, but the mutation renders the Igf2 protein defective. Therefore, the offspring on the right has a dwarf phenotype. As shown here, both offspring have the same genotype; they are heterozygous for the *Igf2* alleles (i.e., *Igf2* $Igf2^{-}$). They are phenotypically different, however, because only the paternally inherited allele is expressed.

At the cellular level, imprinting is an epigenetic process that can be divided into three stages: (1) the establishment of the imprint during gametogenesis, (2) the maintenance of the



INTERACTIVE EXERCISE

FIGURE 5.9 An example of genomic imprinting in the mouse. In the cross on the left, a homozygous male with the normal *Igf2* allele is crossed to a homozygous female carrying a defective allele, designated *Igf2*$^{-}$. An offspring is heterozygous and normal because the paternal allele is active. In the reciprocal cross on the right, a homozygous male carrying the defective allele is crossed to a homozygous normal female. In this case, the offspring is heterozygous and dwarf. This is because the paternal allele is defective due to mutation and the maternal allele is not expressed. The photograph shows normal-size (left) and dwarf littermates (right) derived from a cross between a wild-type female and a heterozygous male carrying a loss-of-function *Igf2* allele (courtesy of A. Efstratiadis). The loss-of-function allele was created using gene knockout methods described in Chapter 19 (see Figure 19.7).

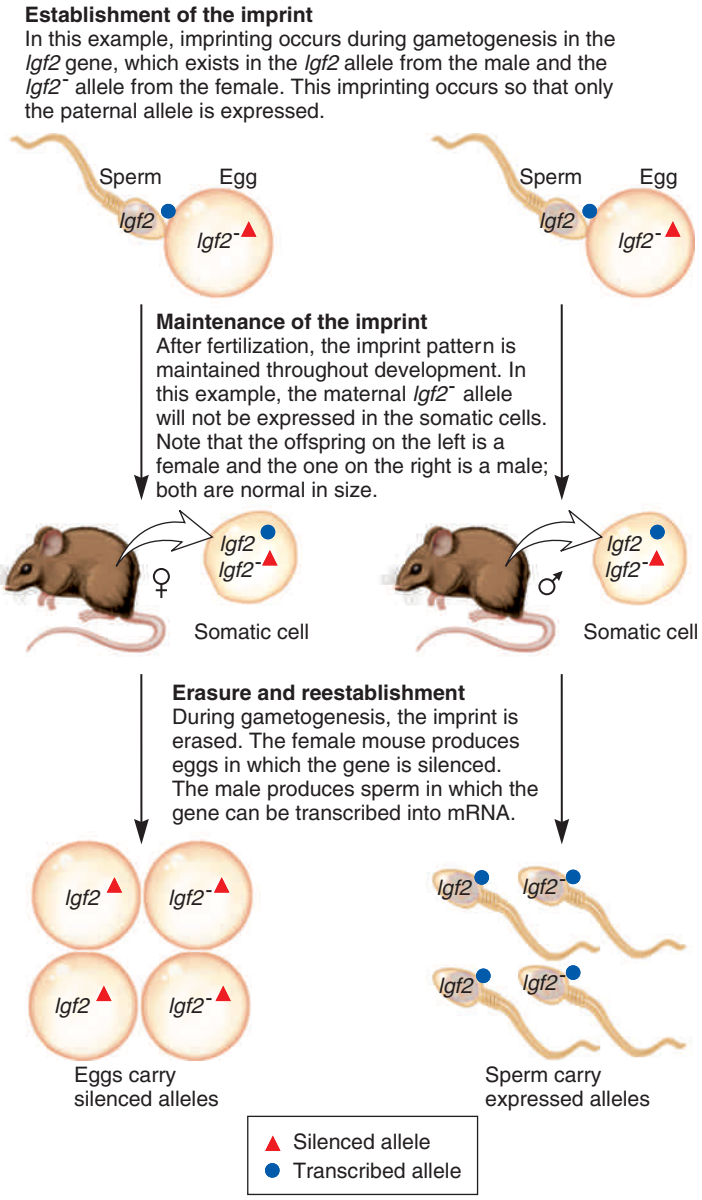


FIGURE 5.10 Genomic imprinting during gametogenesis. This example involves a mouse gene *Igf2*, which is found in two alleles designated *Igf2* and *Igf2*$^{-}$. The left side shows a female mouse that was produced from a sperm carrying the *Igf2* allele and an egg carrying the *Igf2*$^{-}$ allele. In the somatic cells of this female animal, the *Igf2* allele is active. However, when this female produces eggs, both alleles are transcriptionally inactive when they are transmitted to offspring. The right side of this figure shows a male mouse that was also produced from a sperm carrying the *Igf2* allele and an egg carrying the *Igf2*$^{-}$ allele. In the somatic cells of this male animal, the *Igf2* allele is active. However, the sperm from this male contains either a functionally active *Igf2* allele or a functionally defective *Igf2*$^{-}$ allele.

imprint during embryogenesis and in adult somatic cells, and (3) the erasure and reestablishment of the imprint in the germ cells. These stages are described in **Figure 5.10**, which shows the imprinting of the *Igf2* gene. The two mice shown here have inherited the *Igf2* allele from their father and the *Igf2*$^{-}$ allele from their mother. Due to imprinting, both mice express the *Igf2* allele in their somatic cells, and the pattern of imprinting is maintained in the somatic cells throughout development. In the germ cells (i.e., sperm and eggs), the imprint is erased; it will be reestablished according to the sex of the animal. The female mouse on the left will transmit only transcriptionally inactive alleles to her offspring. The male mouse on the right will transmit transcriptionally active alleles. However, because this male is a heterozygote, it will transmit either a functionally active *Igf2* allele or a functionally defective mutant allele (*Igf2*$^{-}$). An *Igf2*$^{-}$ allele, which is inherited from a male mouse, can be expressed into mRNA (i.e., it is transcriptionally active), but it will not produce a functional Igf2 protein due to the deleterious mutation that created the *Igf2*$^{-}$ allele; a dwarf phenotype will result.

As seen in Figure 5.10, genomic imprinting is permanent in the somatic cells of an animal, but the marking of alleles can

be altered from generation to generation. For example, the female mouse on the left possesses an active copy of the *Igf2* allele, but any allele this female transmits to its offspring will be transcriptionally inactive.

Genomic imprinting occurs in several species, including numerous insects, mammals, and flowering plants. Imprinting may involve a single gene, a part of a chromosome, an entire chromosome, or even all of the chromosomes from one parent. Helen Crouse discovered the first example of imprinting, which involved an entire chromosome in the housefly, *Sciara coprophila*. In this species, the fly normally inherits three sex chromosomes, rather than two as in most other species. One X chromosome is inherited from the female, and two are inherited from the male. In male flies, both paternal X chromosomes are lost from somatic cells during embryogenesis. In female flies, only one of the paternal X chromosomes is lost. In both sexes, the maternally inherited X chromosome is never lost. These results indicate that the maternal X chromosome is marked to promote its retention or paternal X chromosomes are marked to promote their loss.

Genomic imprinting can also be involved in the process of X inactivation, described previously. In certain species, imprinting plays a role in the choice of the X chromosome that will be inactivated. For example, in marsupials, the paternal X chromosome is marked so that it is the X chromosome that is always inactivated in the somatic cells of females. In marsupials, X inactivation is not random; the maternal X chromosome is always active.

The Imprinting of Genes and Chromosomes Is a Molecular Marking Process That Involves DNA Methylation

As we have seen, genomic imprinting must involve a marking process. A particular gene or chromosome must be marked differently during spermatogenesis versus oogenesis. After fertilization takes place, this differential marking affects the expression of particular genes. What is the molecular explanation for genomic imprinting? As discussed in Chapter 15, **DNA methylation**—the attachment of a methyl group onto a cytosine base—is a common way that eukaryotic genes may be regulated. Research indicates that genomic imprinting involves an **imprinting control region (ICR)** that is located near the imprinted gene. A portion of the DNA in this region is called the differentially methylated domain (DMD). Depending on the particular gene, the DMD is methylated in the egg or the sperm, but not both. The ICR also contains binding sites for one or more proteins that regulate the transcription of the imprinted gene.

For most imprinted genes, methylation causes an inhibition of gene expression. Methylation could enhance the binding of proteins that inhibit transcription or inhibit the binding of proteins that enhance transcription (or both). (The relationship between methylation and gene expression is described in Chapter 15.) For this reason, imprinting is usually described as a marking process that silences gene expression by preventing transcription. However, this is not always the case. Two imprinted human genes, *H19* and *Igf2*, provide an interesting example. These two genes lie close to each other on human chromosome 11 and appear to be controlled by the same ICR, which is a 52,000-base pair (bp) region that lies between the *Igf2* and *H19* genes (**Figure 5.11**). It contains binding sites for proteins that regulate the transcription of the *H19* or *Igf2* genes. This ICR is highly methylated on the paternally inherited chromosome but not on the maternally inherited one.

When the ICR is not methylated, a protein called CTC-binding factor is able to bind to the ICR (Figure 5.11a). The CTC-binding factor gets its name from the observation that it binds to a region of DNA that is rich in CTC (cytosine-thymine-cytosine) sequences. The ICR contains several such CTC sequences. When they are unmethylated, the CTC-binding factor can bind to the ICR. As described in Figure 5.11a, this has two effects. First, it prevents the binding of activator proteins to the *Igf2* gene, thereby shuting off this gene. In contrast, it permits activator proteins to turn on the *H19* gene.

How does methylation affect the transcription of the *Igf2* and *H19* genes? When the cytosines within the ICR become methylated, the CTC-binding factor is unable to bind to the ICR (Figure 5.11b). This permits activator proteins to turn on the *Igf2* gene. The DNA methylation also causes the repression of the *H19* gene so it is not transcribed.

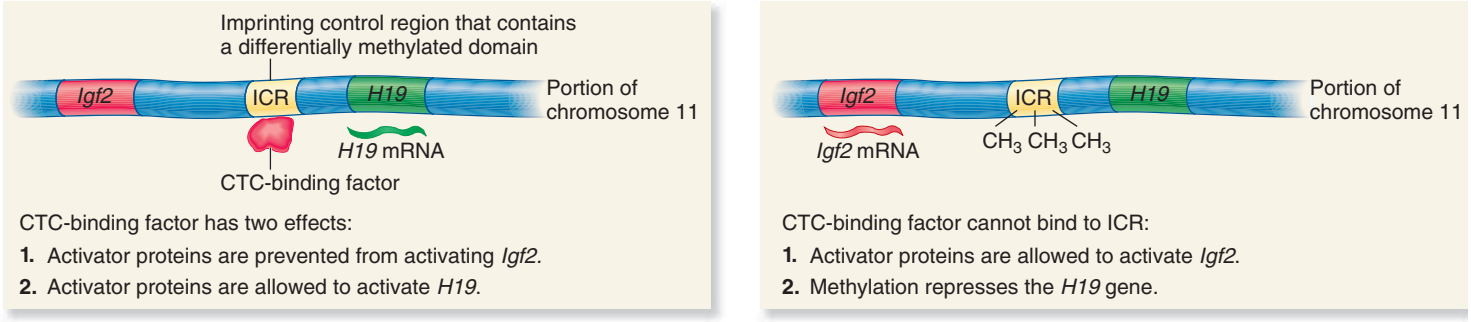


FIGURE 5.11 **A simplified scheme for how DNA methylation at the ICR affects the expression of the *Igf2* and *H19* genes.** **(a)** The lack of methylation of the maternal chromosome causes the *Igf2* gene to be turned off and the *H19* gene to be turned on. **(b)** The methylation of the paternal chromosome has the opposite effect. The *Igf2* gene is turned on and the *H19* gene is turned off.

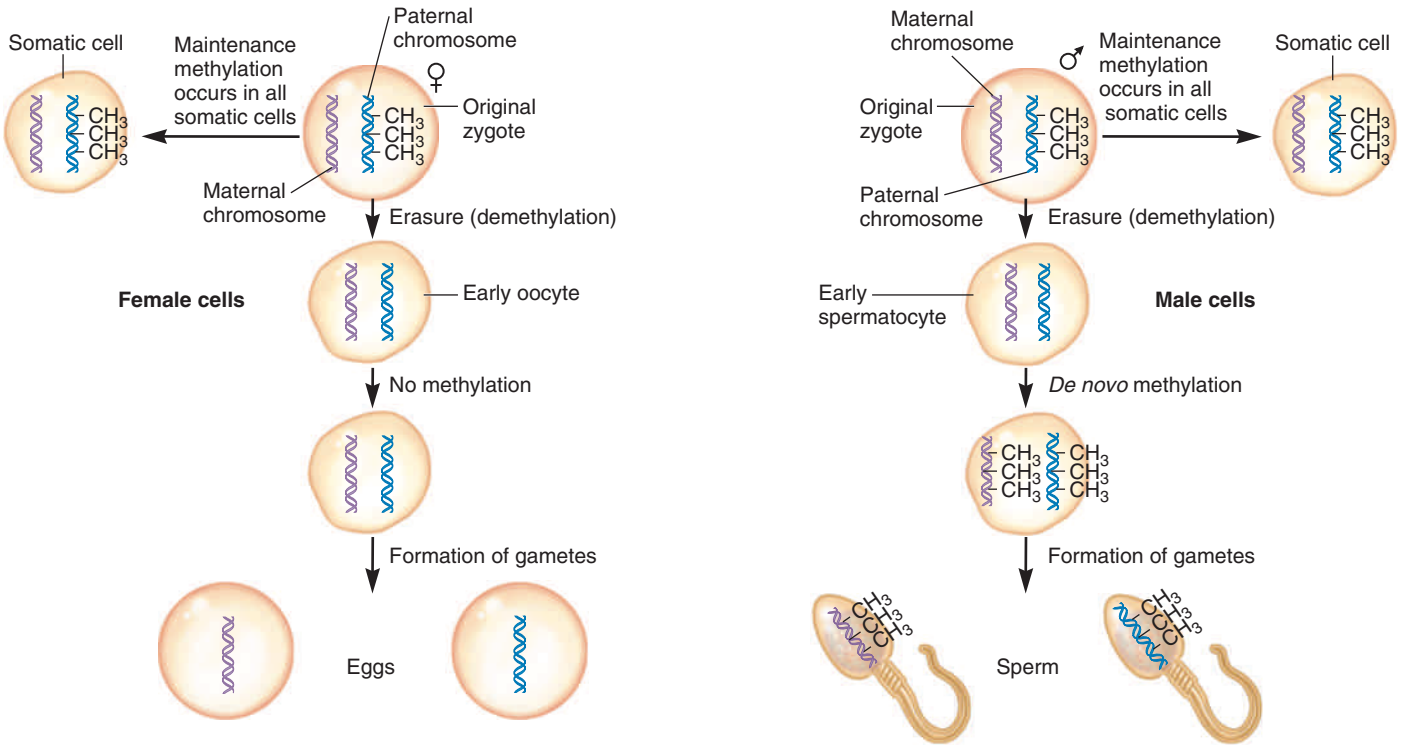


FIGURE 5.12 **The pattern of ICR methylation from one generation to the next.** In this example, a male and a female offspring have inherited a methylated ICR and nonmethylated ICR from their father and mother, respectively. Maintenance methylation retains the imprinting in somatic cells during embryogenesis and in adulthood. Demethylation occurs in cells that are destined to become gametes. In this example, *de novo* methylation occurs only in cells that are destined to become sperm. Haploid male gametes transmit a methylated ICR, whereas haploid female gametes transmit an unmethylated ICR.

Imprinting from Generation to Generation Involves Maintenance, Erasure, and *De Novo* Methylation Steps

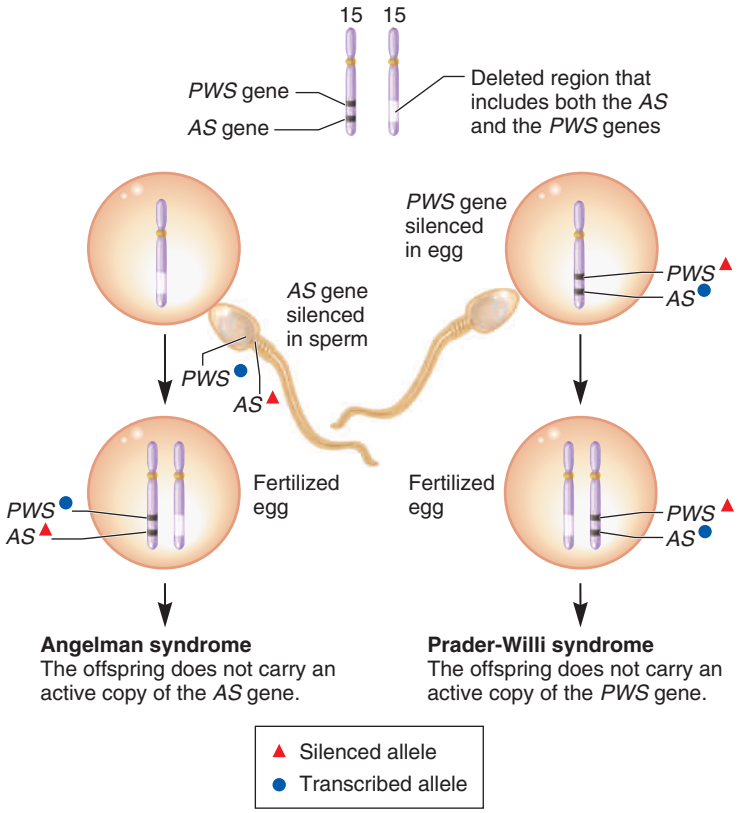
Now that we have an understanding of how methylation may affect gene transcription, let's consider the methylation process from one generation to the next. In the example shown in **Figure 5.12**, the paternally inherited allele for a particular gene is methylated but the maternally inherited allele is not. A female (left side) and male (right side) have inherited a methylated ICR from their father and an unmethylated ICR from their mother. This pattern of imprinting is maintained in the somatic cells of both individuals. However, when the female makes gametes, the imprinting is erased during early oogenesis, so the female will pass an unmethylated ICR to its offspring. In the male, the imprinting is also erased during early spermatogenesis, but then *de novo* (new) methylation occurs in both ICRs. Therefore, the male will transmit a methylated gene to its offspring.

Imprinting Plays a Role in the Inheritance of Certain Human Genetic Diseases

Human diseases, such as Prader-Willi syndrome (PWS) and Angelman syndrome (AS), are influenced by imprinting. PWS is characterized by reduced motor function, obesity, and small hands and feet. Individuals with AS are thin and hyperactive, have unusual seizures and repetitive symmetrical muscle movements, and exhibit mental deficiencies. Most commonly, both PWS and AS involve a small deletion in human chromosome 15. If this deletion is inherited from the mother, it leads to Angelman syndrome; if inherited from the father, it leads to Prader-Willi syndrome (**Figure 5.13**).

Researchers have discovered that this region of chromosome 15 contains closely linked but distinct genes that are maternally or paternally imprinted. AS results from the lack of expression of a single gene (*UBE3A*) that codes for a protein called E6-AP, which functions to transfer small ubiquitin molecules to certain proteins to target their degradation. Both copies of this gene are active in many of the body's tissues. In the brain, however, only the copy inherited from a person's mother (the maternal copy) is active. The paternal allele of *UBE3A* is silenced. Therefore, if the maternal allele is deleted, as in the left side of Figure 5.13, the individual will develop AS because she or he will not have an active copy of the *UBE3A* gene.

The gene(s) responsible for PWS has not been definitively determined, although five imprinted genes in this region of chromosome 15 are known. One possible candidate involved in PWS is a gene designated *SNRPN*. The gene product is part of a small nuclear ribonucleoprotein polypeptide N, which is a complex that controls RNA splicing and is necessary for the synthesis of critical proteins in the brain. The maternal allele of *SNRPN* is silenced, and only the paternal copy is active.



INTERACTIVE EXERCISE

FIGURE 5.13 The role of imprinting in the development of Angelman and Prader-Willi syndromes.

Genes → Traits A small region on chromosome 15 contains two different genes designated the *AS* gene and *PWS* gene in this figure. If a chromosome 15 deletion is inherited from the mother, Angelman syndrome occurs because the offspring does not inherit an active copy of the *AS* gene (left). Alternatively, the chromosome 15 deletion may be inherited from the father, leading to Prader-Willi syndrome. The phenotype of this syndrome occurs because the offspring does not inherit an active copy of the *PWS* gene (right).

The Biological Significance of Imprinting Is Not Well Understood

Genomic imprinting is a fairly new and exciting area of research. Imprinting has been identified in many mammalian genes (Table 5.2). In some cases, the female alleles are transcriptionally active in the somatic cells of offspring, whereas in other cases, the male alleles are active.

The biological significance of imprinting is still a matter of much speculation. Several hypotheses have been proposed to explain the potential benefits of genomic imprinting. One example, described by David Haig, involves differences in female versus male reproductive patterns in mammals. As discussed in Chapter 24, natural selection favors types of genetic variation that confer a reproductive advantage. The likelihood that favorable variation will be passed to offspring may differ between the sexes. In many mammalian species, females may mate with multiple males, perhaps generating embryos in the same uterus fathered by different males. For males, silencing genes that inhibit embryonic growth would be an advantage. The embryos of males that silence such genes would grow faster than other embryos in the same uterus, making it more likely for the males to pass their genes to future generations. For females, however, rapid growth of embryos might be a disadvantage because it could drain too many resources from the mother. According to this scenario, the mother would silence genes that cause rapid embryonic growth. From the mother's perspective, she would give all of her offspring an equal chance of survival without sapping her own strength. This would make it more likely for the female to pass her genes to future generations.

The Haig hypothesis seems to be consistent with the imprinting of several mammalian genes that are involved with growth, such as *Igf2*. Females silence this growth-enhancing gene, whereas males do not. However, several imprinted genes do not seem to play a role in embryonic development. Therefore, an understanding of the biological role(s) of genomic imprinting requires further investigation.

TABLE 5.2

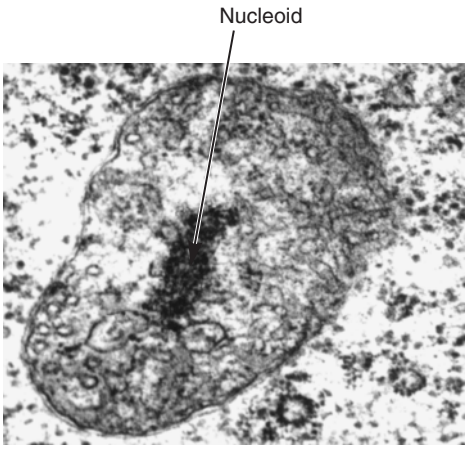
Examples of Mammalian Genes and Inherited Human Diseases That Involve Imprinted Genes*

Gene	Allele Expressed	Function
WT1	Maternal	Wilms tumor–suppressor gene; suppresses cell growth
INS	Paternal	Insulin; hormone involved in cell growth and metabolism
Igf2	Paternal	Insulin-like growth factor II; similar to insulin
Igf2R	Maternal	Receptor for insulin-like growth factor II
H19	Maternal	Unknown
SNRPN	Paternal	Splicing factor
Gabrb	Maternal	Neurotransmitter receptor

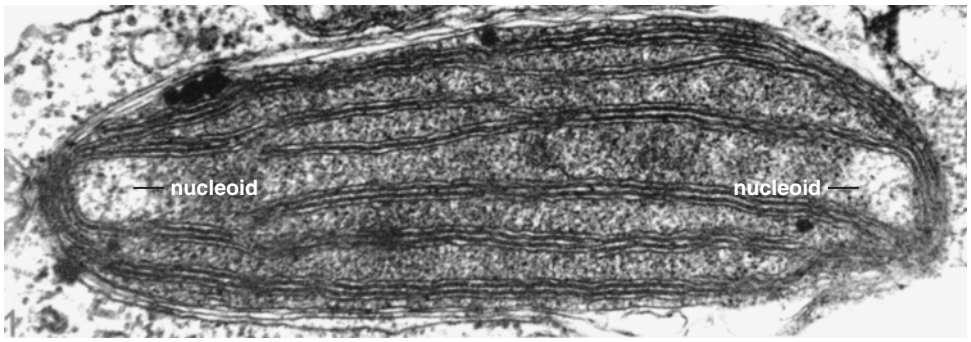
*Researchers estimate that approximately 1–2% of human genes are subjected to genomic imprinting, but fewer than 100 have actually been demonstrated to be imprinted.

5.3 EXTRANUCLEAR INHERITANCE

Thus far, we have considered several types of non-Mendelian inheritance patterns. These include maternal effect genes, dosage compensation, and genomic imprinting. All of these inheritance patterns involve genes found on chromosomes in the cell nucleus. Another cause of non-Mendelian inheritance patterns involves genes that are not located in the cell nucleus. In eukaryotic species, the most biologically important example of extranuclear inheritance involves genetic material in cellular organelles. In addition to the cell nucleus, the mitochondria and chloroplasts contain their own genetic material. Because these organelles are found within the cytoplasm of the cells, the inheritance of organellar genetic material is called **extranuclear inheritance** (the prefix *extra-* means outside of) or **cytoplasmic inheritance.** In this section, we will examine the genetic composition of mitochondria and chloroplasts and explore the pattern



(a) Mitochondrion



(b) Chloroplast

FIGURE 5.14 **Nucleoids within (a) a mitochondrion and (b) a chloroplast.** The mitochondrial and chloroplast chromosomes are found within the nucleoid region of the organelle.

of transmission of these organelles from parent to offspring. We will also consider a few other examples of inheritance patterns that cannot be explained by the transmission of nuclear genes.

Mitochondria and Chloroplasts Contain Circular Chromosomes with Many Genes

In 1951, Yukako Chiba was the first to suggest that chloroplasts contain their own DNA. He based his conclusion on the staining properties of a DNA-specific dye known as Feulgen. Researchers later developed techniques to purify organellar DNA. In addition, electron microscopy studies provided interesting insights into the organization and composition of mitochondrial and chloroplast chromosomes. More recently, the advent of molecular genetic techniques in the 1970s and 1980s has allowed researchers to determine the genome sequences of organellar DNAs. From these types of studies, the chromosomes of mitochondria and chloroplasts were found to resemble smaller versions of bacterial chromosomes.

The genetic material of mitochondria and chloroplasts is located inside the organelle in a region known as the **nucleoid** (**Figure 5.14**). The genome is a single circular chromosome (composed of double-stranded DNA), although a nucleoid contains several copies of this chromosome. In addition, a mitochondrion or chloroplast often has more than one nucleoid. In mice, for example, each mitochondrion has one to three nucleoids, with each nucleoid containing two to six copies of the circular mitochondrial genome. However, this number varies depending on the type of cell and the stage of development. In comparison, the chloroplasts of algae and higher plants tend to have more nucleoids per organelle. **Table 5.3** describes the genetic composition of mitochondria and chloroplasts for a few selected species.

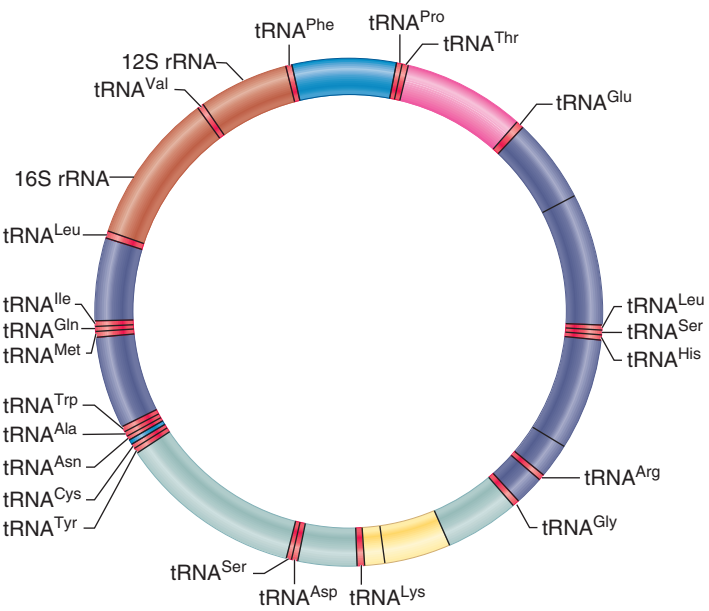
Besides variation in copy number, the sizes of mitochondrial and chloroplast genomes also vary greatly among different species. For example, a 400-fold variation is found in the sizes of mitochondrial chromosomes. In general, the mitochondrial genomes of animal species tend to be fairly small; those of fungi and protists are intermediate in size; and those of plant cells tend to be fairly large. Among algae and plants, substantial variation is also found in the sizes of chloroplast chromosomes.

TABLE 5.3
Genetic Composition of Mitochondria and Chloroplasts

Species	Organelle	Nucleoids per Organelle	Total Number of Chromosomes per Organelle
Tetrahymena	Mitochondrion	1	6–8
Mouse	Mitochondrion	1–3	5–6
Chlamydomonas	Chloroplast	5–6	~80
Euglena	Chloroplast	20–34	100–300
Higher plants	Chloroplast	12–25	~60

Data from: Gillham, N. W. (1994). *Organelle Genes and Genomes.* Oxford University Press, New York.

Figure 5.15 illustrates a map of human **mitochondrial DNA (mtDNA).** Each copy of the mitochondrial chromosome consists of a circular DNA molecule that is only 17,000 bp in length. This size is less than 1% of a typical bacterial chromosome. The human mtDNA carries relatively few genes. Thirteen genes encode proteins that function within the mitochondrion. In addition, mtDNA carries genes that encode ribosomal RNA and transfer RNA. These rRNAs and tRNAs are necessary for the synthesis of the 13 polypeptides that are encoded by the mtDNA. The primary role of mitochondria is to provide cells with the bulk of their adenosine triphosphate (ATP), which is used as an energy source to drive cellular reactions. These 13 polypeptides are subunits of proteins that function in a process known as oxidative phosphorylation, in which mitochondria use oxygen and synthesize ATP. However, mitochondria require many additional proteins to carry out oxidative phosphorylation and



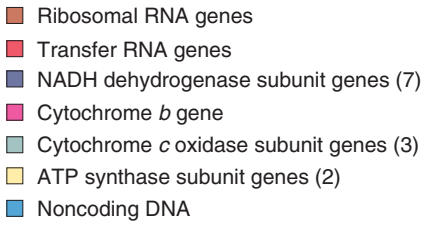


FIGURE 5.15 A genetic map of human mitochondrial DNA (mtDNA). This diagram illustrates the locations of many genes along the circular mitochondrial chromosome. The genes shown in red encode transfer RNAs. For example, $tRNA^{Arg}$ encodes a tRNA that carries arginine. The genes that encode ribosomal RNA are shown in light brown. The remaining genes encode proteins that function within the mitochondrion. The mitochondrial genomes from numerous species have been determined.

other mitochondrial functions. Most mitochondrial proteins are encoded by genes within the cell nucleus. When these nuclear genes are expressed, the mitochondrial polypeptides are first synthesized outside the mitochondria in the cytosol of the cell. They are then transported into the mitochondria where they may associate with other polypeptides and become functional proteins.

Chloroplast genomes tend to be larger than mitochondrial genomes, and they have a correspondingly greater number of genes. A typical chloroplast genome is approximately 100,000 to 200,000 bp in length, which is about 10 times larger than the mitochondrial genome of animal cells. Figure 5.16 shows the **chloroplast DNA (cpDNA)** of the tobacco plant, which is

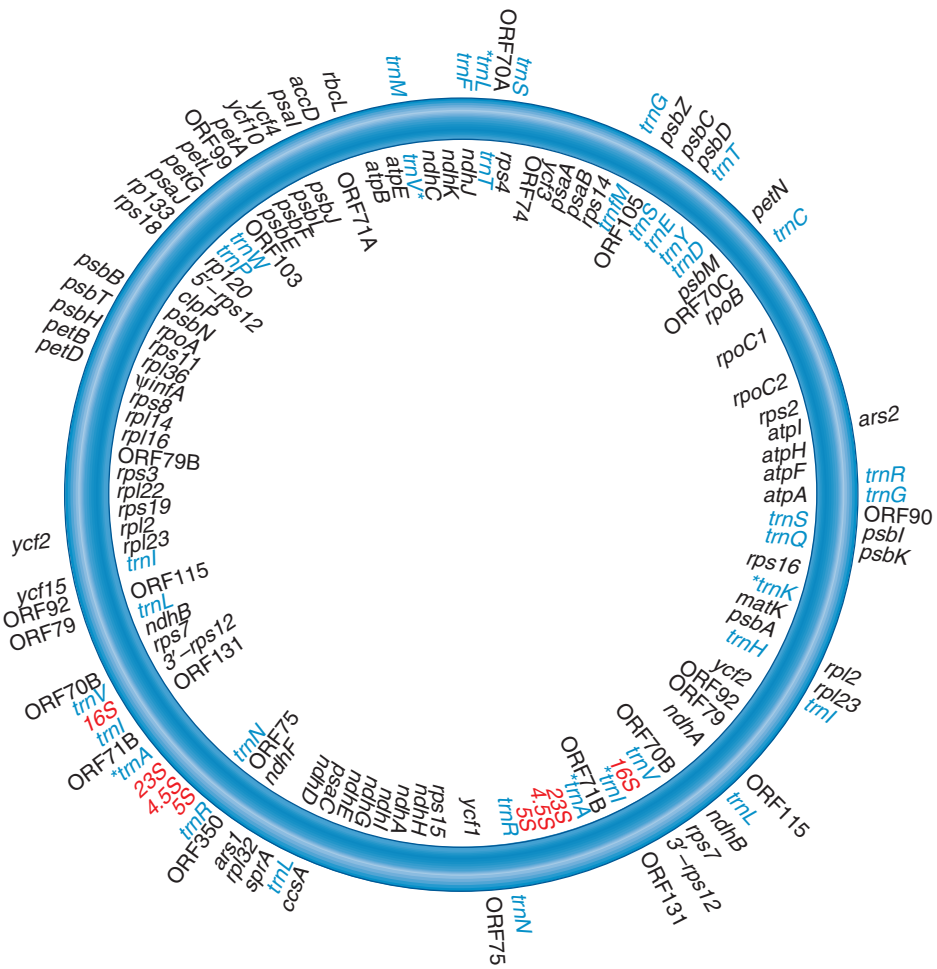


FIGURE 5.16 A genetic map of tobacco chloroplast DNA (cpDNA). This diagram illustrates the locations of many genes along the circular chloroplast chromosome. The gene names shown in blue encode transfer RNAs. The genes that encode ribosomal RNA are shown in red. The remaining genes shown in black encode polypeptides that function within the chloroplast. The genes designated ORF (open reading frame) encode polypeptides with unknown functions.

a circular DNA molecule that contains 156,000 bp of DNA and carries between 110 and 120 different genes. These genes encode ribosomal RNAs, transfer RNAs, and many proteins required for photosynthesis. As with mitochondria, many chloroplast proteins are encoded by genes found in the plant cell nucleus. These proteins contain chloroplast-targeting signals that direct them into the chloroplasts.

Extranuclear Inheritance Produces Non-Mendelian Results in Reciprocal Crosses

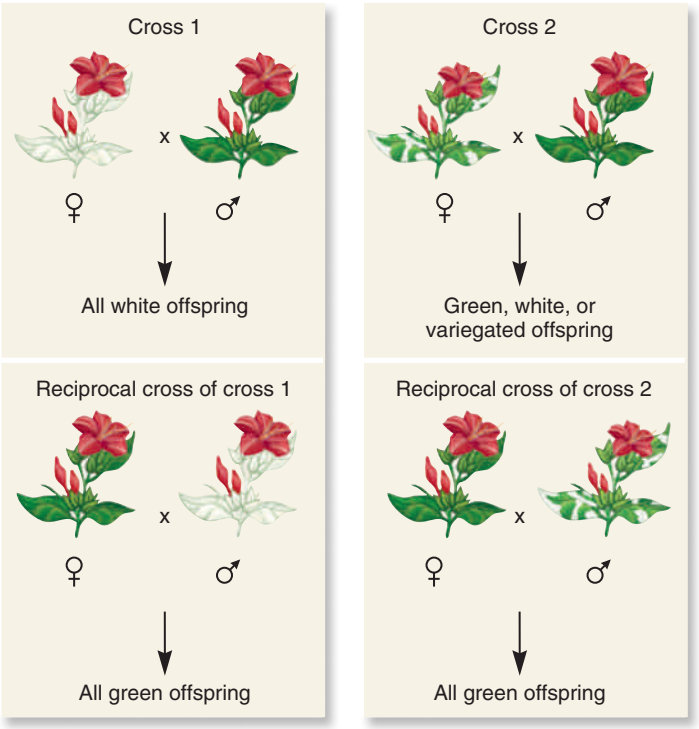
In diploid eukaryotic species, most genes within the nucleus obey a Mendelian pattern of inheritance because the homologous pairs of chromosomes segregate during gamete formation. Except for sex-linked traits, offspring inherit one copy of each gene from both the maternal and paternal parents. The sorting of chromosomes during meiosis explains the inheritance patterns of nuclear genes. By comparison, the inheritance of extranuclear genetic material does not display a Mendelian pattern. Mitochondria and chloroplasts are not sorted during meiosis and therefore do not segregate into gametes in the same way as nuclear chromosomes.

In 1909, Carl Correns discovered a trait that showed a non-Mendelian pattern of inheritance involving pigmentation in *Mirabilis jalapa* (the four-o'clock plant). Leaves can be green, white, or variegated with both green and white sectors. Correns demonstrated that the pigmentation of the offspring depended solely on the maternal parent (**Figure 5.17**). If the female parent had white pigmentation, all offspring had white leaves. Similarly, if the female was green, all offspring were green. When the female was variegated, the offspring could be green, white, or variegated.

The pattern of inheritance observed by Correns is a type of extranuclear inheritance called **maternal inheritance** (not to be confused with maternal effect). Chloroplasts are a type of plastid that makes chlorophyll, a green photosynthetic pigment. Maternal inheritance occurs because the chloroplasts are inherited only through the cytoplasm of the egg. The pollen grains of *M. jalapa* do not transmit chloroplasts to the offspring.

The phenotypes of leaves can be explained by the types of chloroplasts within the leaf cells. The green phenotype, which is the wild-type condition, is due to the presence of normal chloroplasts that make green pigment. By comparison, the white phenotype is due to a mutation in a gene within the chloroplast DNA that diminishes the synthesis of green pigment. A cell may contain both types of chloroplasts, a condition known as **heteroplasmy.** A leaf cell containing both types of chloroplasts is green because the normal chloroplasts produce green pigment.

How does a variegated phenotype occur? **Figure 5.18** considers the leaf of a plant that began from a fertilized egg that contained both types of chloroplasts (i.e., a heteroplasmic cell). As a plant grows, the two types of chloroplasts are irregularly distributed to daughter cells. On occasion, a cell may receive only the chloroplasts that have a defect in making green pigment. Such a cell continues to divide and produce a sector of the plant that is entirely white. In this way, the variegated phenotype is produced. Similarly, if we consider the results of Figure 5.17, a female parent that is variegated may transmit green, white, or a mixture of these types of chloroplasts to the egg cell, thereby producing green, white, or variegated offspring, respectively.



INTERACTIVE EXERCISE

FIGURE 5.17 Maternal inheritance in the four-o'clock plant, *Mirabilis jalapa*. The reciprocal crosses of four-o'clock plants by Carl Correns consisted of a pair of crosses between white-leaved and green-leaved plants, and a pair of crosses between variegated-leaved and green-leaved plants.

Genes → Traits In this example, the white phenotype is due to chloroplasts that carry a mutant allele that diminishes green pigmentation. The variegated phenotype is due to a mixture of chloroplasts, some of which carry the normal (green) allele and some of which carry the white allele. In the crosses shown here, the parent providing the eggs determines the phenotypes of the offspring. This is due to maternal inheritance. The egg contains the chloroplasts that are inherited by the offspring. (Note: The defective chloroplasts that give rise to white sectors are not completely defective in chlorophyll synthesis. Therefore, entirely white plants can survive, though they are smaller than green or variegated plants.)

Studies in Yeast and *Chlamydomonas* Provided Genetic Evidence for Extranuclear Inheritance of Mitochondria and Chloroplasts

The research of Correns and others indicated that some traits, such as leaf pigmentation, are inherited in a non-Mendelian manner. However, such studies did not definitively determine that maternal inheritance is due to genetic material within organelles. Further progress in the investigation of extranuclear inheritance was provided by detailed genetic analyses of eukaryotic microorganisms, such as yeast and algae, by isolating and characterizing mutant phenotypes that specifically affected the chloroplasts or mitochondria.

During the 1940s and 1950s, yeasts and molds became model eukaryotic organisms for investigating the inheritance of mitochondria. Because mitochondria produce energy for cells in

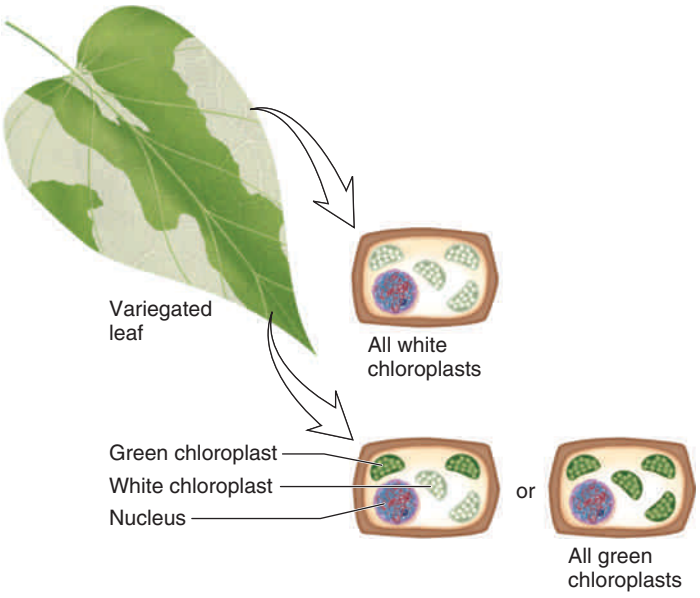


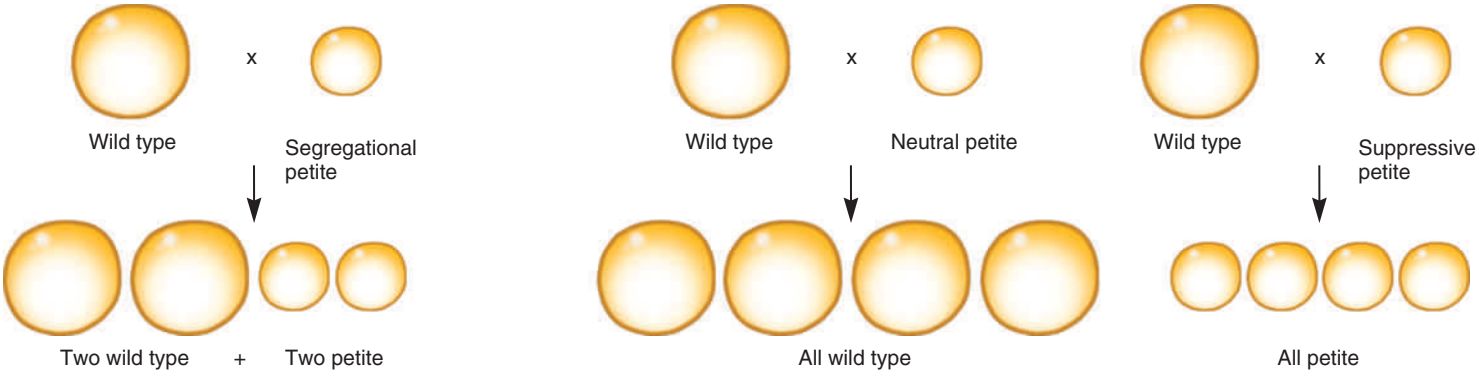
FIGURE 5.18 A cellular explanation of the variegated phenotype in *Mirabilis jalapa*. This plant inherited two types of chloroplasts—those that can produce green pigment and those that are defective. As the plant grows, the two types of chloroplasts are irregularly distributed to daughter cells. On occasion, a leaf cell may receive only the chloroplasts that are defective at making green pigment. Such a cell continues to divide and produces a sector of the leaf that is entirely white. Cells that contain both types of chloroplasts or cells that contain only green chloroplasts produce green tissue, which may be adjacent to a sector of white tissue. This is the basis for the variegated phenotype of the leaves.

the form of ATP, mutations that yield defective mitochondria are expected to make cells grow much more slowly. Boris Ephrussi and his colleagues identified mutations in *Saccharomyces cerevisiae* that had such a phenotype. These mutants were called **petites** to describe their formation of small colonies on agar plates as opposed to wild-type strains that formed larger colonies. Biochemical and physiological evidence indicated that petite mutants had defective mitochondria. The researchers found that petite mutants could not grow when the cells had an energy source requiring only the metabolic activity of mitochondria, but could form small colonies when grown on sugars metabolized by the glycolytic pathway, which occurs outside the mitochondria.

Because yeast cells exist in two mating types, designated a and α, Ephrussi was able to mate a wild-type strain to his petite mutants. Genetic analyses showed that petite mutants were inherited in different ways. When a wild-type strain was crossed to a segregational petite mutant, he obtained a ratio of 2 wild-type cells to 2 petite cells (**Figure 5.19a**). This result is consistent with a Mendelian pattern of inheritance (see the discussion of tetrad analysis in Chapter 6). Therefore, segregational petite mutations cause defects in genes located in the cell nucleus. These genes encode proteins necessary for mitochondrial function. Such proteins are synthesized in the cytosol and are then taken up by the mitochondria, where they perform their functions. Segregational petites get their name because they segregate in a Mendelian manner during meiosis.

By comparison, the second category of petite mutants, known as vegetative petite mutants, did not segregate in a Mendelian manner (**Figure 5.19b**). Ephrussi identified two types of vegetative petites, called neutral petites and suppressive petites. In a cross between a wild-type strain and a neutral petite, all four haploid daughter cells were wild type. This type of inheritance contradicts the normal 2:2 ratio expected for the segregation of Mendelian traits. In comparison, a cross between a wild-type strain and a suppressive petite usually yielded all petite colonies. Thus, both types of vegetative petites are defective in mitochondrial function and show a non-Mendelian pattern of inheritance. These results occurred because vegetative petites carry mutations in the mitochondrial genome itself.

Since these initial studies, researchers have found that neutral petites lack most of their mitochondrial DNA, whereas suppressive petites usually lack small segments of the mitochondrial genetic material. When two yeast cells are mated, the daughter cells inherit mitochondria from both parents. For example, in a cross between a wild-type and a neutral petite strain, the daughter cells inherit both types of mitochondria. Because wild-type



(a) A cross between a wild type and a segregational petite

(b) A cross between a wild type and a neutral or suppressive vegetative petite

INTERACTIVE EXERCISE

FIGURE 5.19 Transmission of the petite trait in *Saccharomyces cerevisiae*. **(a)** A wild-type strain crossed to a segregational petite. **(b)** A wild-type strain crossed to a neutral vegetative petite and to a suppressive vegetative petite.

mitochondria are inherited, the cells display a normal phenotype. The inheritance pattern of suppressive petites is more difficult to explain because the daughter cells inherit both normal and suppressive petite mitochondria. One possibility is that the suppressive petite mitochondria replicate more rapidly so that the wild-type mitochondria are not maintained in the cytoplasm for many doublings. Alternatively, experimental evidence suggests that genetic exchanges between the mitochondrial genomes of wild-type and suppressive petites may ultimately produce a defective population of mitochondria.

Let's now turn our attention to the inheritance of chloroplasts that are found in eukaryotic species capable of photosynthesis (namely, algae and plants). The unicellular alga *Chlamydomonas reinhardtii* is used as a model organism to investigate the inheritance of chloroplasts. This organism contains a single chloroplast that occupies approximately 40% of the cell volume. Genetic studies of chloroplast inheritance began when Ruth Sager identified a mutant strain of *Chlamydomonas* that is resistant to the antibiotic streptomycin (sm^r). By comparison, most strains are sensitive to killing by streptomycin (sm^s).

Sager conducted crosses to determine the inheritance pattern of the sm^r gene. During mating, two haploid cells unite to form a diploid cell, which then undergoes meiosis to form four haploid cells. Like yeast, *Chlamydomonas* is an organism that can be found in two mating types, in this case, designated mt^+ and mt^-. Mating type is due to nuclear inheritance and segregates in a 1:1 manner. By comparison, Sager and her colleagues discovered that the sm^r gene was inherited from the mt^+ parent but not from the mt^- parent (**Figure 5.20**). Therefore, this sm^r gene was not inherited in a Mendelian manner. This pattern occurred because only the mt^+ parent transmits chloroplasts to daughter cells and the sm^r gene is found in the chloroplast genome.

The Pattern of Inheritance of Mitochondria and Chloroplasts Varies Among Different Species

The inheritance of traits via genetic material within mitochondria and chloroplasts is now a well-established phenomenon that geneticists have investigated in many different species. In **heterogamous** species, two kinds of gametes are made. The female gamete tends to be large and provides most of the cytoplasm to the zygote, whereas the male gamete is small and often provides little more than a nucleus. Therefore, mitochondria and chloroplasts are most often inherited from the maternal parent. However, this is not always the case. **Table 5.4** describes the inheritance patterns of mitochondria and chloroplasts in several selected species.

In species in which maternal inheritance is generally observed, the paternal parent may occasionally provide mitochondria via the sperm. This phenomenon, called **paternal leakage,** occurs in many species that primarily exhibit maternal inheritance of their organelles. In the mouse, for example, approximately one to four paternal mitochondria are inherited for every 100,000 maternal mitochondria per generation of offspring. Most offspring do not inherit any paternal mitochondria, but a rare individual may inherit a mitochondrion from the sperm.

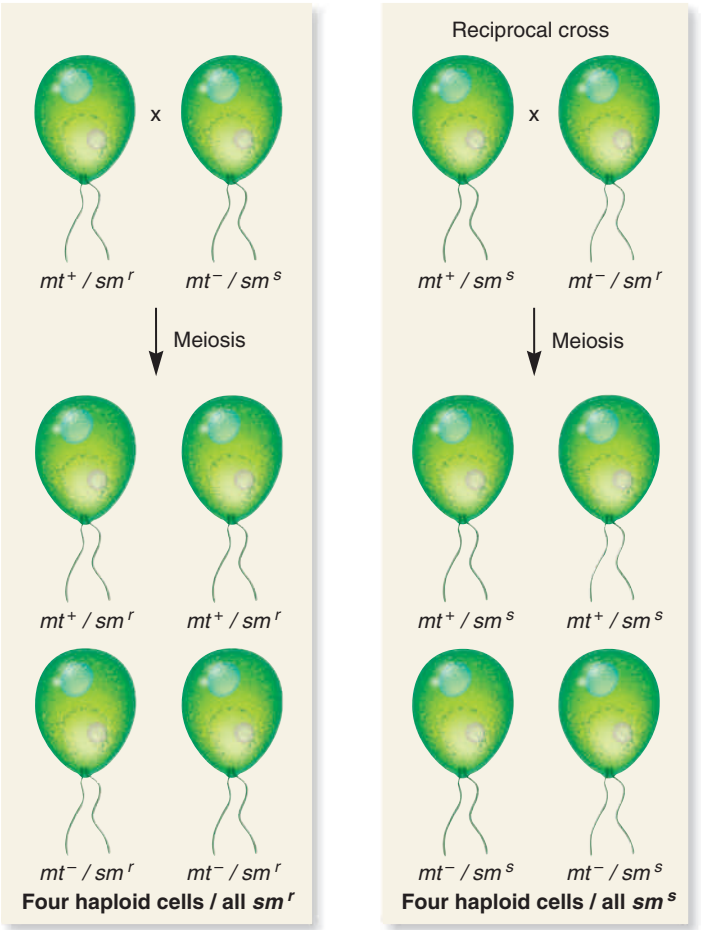


FIGURE 5.20 Chloroplast inheritance in *Chlamydomonas*. The two mating types of the organism are indicated as mt^+ and mt^-. Sm^r indicates streptomycin resistance, whereas sm^s indicates sensitivity to this antibiotic.

TABLE 5.4
Transmission of Organelles Among Different Species

Species	Organelle	Transmission
Mammals	Mitochondria	Maternal inheritance
S. cerevisiae	Mitochondria	Biparental inheritance
Molds	Mitochondria	Usually maternal inheritance; paternal inheritance has been found in the genus *Allomyces*
Chlamydomonas	Mitochondria	Inherited from the parent with the mt^- mating type
Chlamydomonas	Chloroplasts	Inherited from the parent with the mt^+ mating type
Plants		
Angiosperms	Mitochondria and chloroplasts	Often maternal inheritance, although biparental inheritance is found among some species
Gymnosperms	Mitochondria and chloroplasts	Usually paternal inheritance

Many Human Diseases Are Caused by Mitochondrial Mutations

Mitochondrial diseases can occur in two ways. In some cases, mitochondrial mutations that cause disease are transmitted from mother to offspring. Human mtDNA is maternally inherited because it is transmitted from mother to offspring via the cytoplasm of the egg. Therefore, the transmission of inherited human mitochondrial diseases follows a maternal inheritance pattern. In addition, mitochondrial mutations may occur in somatic cells and accumulate as a person ages. Researchers have discovered that mitochondria are particularly susceptible to DNA damage. When more oxygen is consumed than is actually used to make ATP, mitochondria tend to produce free radicals that damage DNA. Unlike nuclear DNA, mitochondrial DNA has very limited repair abilities and almost no protective ability against free radical damage.

Table 5.5 describes several mitochondrial diseases that have been discovered in humans and are caused by mutations in mitochondrial genes. Over 200 diseases associated with defective mitochondria have been discovered. These are usually chronic degenerative disorders that affect cells requiring a high level of ATP, such as nerve and muscle cells. For example, Leber hereditary optic neuropathy (LHON) affects the optic nerve and may lead to the progressive loss of vision in one or both eyes. LHON can be caused by a defective mutation in one of several different mitochondrial genes. Researchers are still investigating how a defect in these mitochondrial genes produces the symptoms of this disease.

An important factor in mitochondrial disease is heteroplasmy, which means that a cell contains a mixed population of mitochondria. Within a single cell, some mitochondria may carry a disease-causing mutation whereas others may not. As cells divide, mutant and normal mitochondria randomly segregate into the resulting daughter cells. Some daughter cells may receive a high ratio of mutant to normal mitochondria, whereas others may receive a low ratio. To cause disease that affects a particular cell or tissue, the ratio of mutant to normal mitochondria must exceed a certain threshold value before disease symptoms are observed.

TABLE **5.5**

Examples of Human Mitochondrial Diseases

Disease	Mitochondrial Gene Mutated
Leber hereditary optic neuropathy	A mutation in one of several mitochondrial genes that encode respiratory chain proteins: *ND1, ND2, CO1, ND4, ND5, ND6,* and *cytb*
Neurogenic muscle weakness	A mutation in the *ATPase6* gene that encodes a subunit of the mitochondrial ATP-synthetase, which is required for ATP synthesis
Mitochondrial encephalomyopathy, lactic acidosis, and strokelike episodes	A mutation in genes that encode tRNAs for leucine and lysine
Mitochondrial myopathy	A mutation in a gene that encodes a tRNA for leucine
Maternal myopathy and cardiomyopathy	A mutation in a gene that encodes a tRNA for leucine
Myoclonic epilepsy with ragged-red muscle fibers	A mutation in a gene that encodes a tRNA for lysine

Extranuclear Genomes of Mitochondria and Chloroplasts Evolved from an Endosymbiotic Relationship

The idea that the nucleus, mitochondria, and chloroplasts contain their own separate genetic material may at first seem puzzling. Wouldn't it be simpler to have all of the genetic material in one place in the cell? The underlying reason for distinct genomes of mitochondria and chloroplasts can be traced back to their evolutionary origin, which is thought to involve a symbiotic association.

A symbiotic relationship occurs when two different species live together in a close association. The symbiont is the smaller of the two species; the host is the larger. The term **endosymbiosis** describes a symbiotic relationship in which the symbiont actually lives inside (*endo-*, inside) the host. In 1883, Andreas Schimper proposed that chloroplasts were descended from an endosymbiotic relationship between cyanobacteria and eukaryotic cells. This idea, now known as the **endosymbiosis theory,** suggested that the ancient origin of chloroplasts was initiated when a cyanobacterium took up residence within a primordial eukaryotic cell (**Figure 5.21**). Over the course of evolution, the

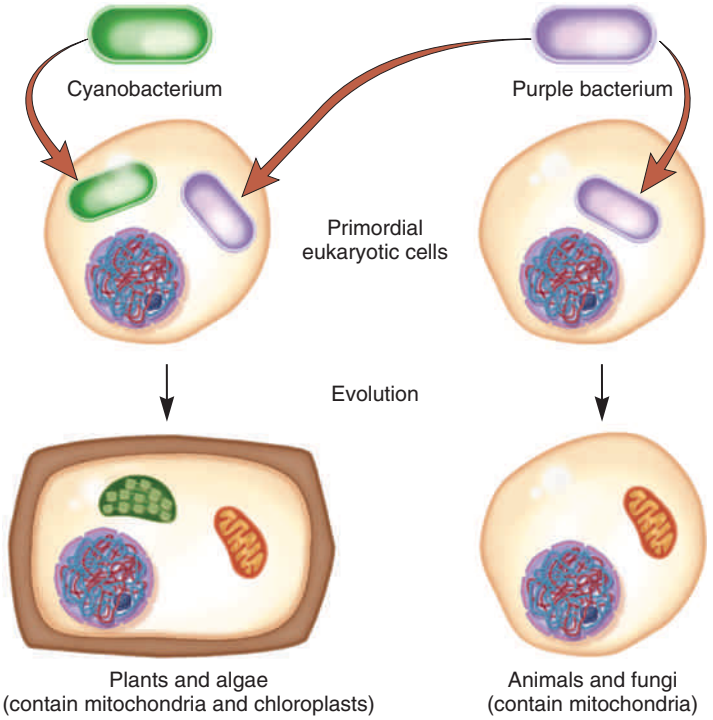


FIGURE 5.21 The endosymbiotic origin of mitochondria and chloroplasts. According to the endosymbiotic theory, chloroplasts descended from an endosymbiotic relationship between cyanobacteria and eukaryotic cells. This arose when a bacterium took up residence within a primordial eukaryotic cell. Over the course of evolution, the intracellular bacterial cell gradually changed its characteristics, eventually becoming a chloroplast. Similarly, mitochondria are derived from an endosymbiotic relationship between purple bacteria and eukaryotic cells.

characteristics of the intracellular bacterial cell gradually changed to those of a chloroplast. In 1922, Ivan Wallin also proposed an endosymbiotic origin for mitochondria.

In spite of these hypotheses, the question of endosymbiosis was largely ignored until researchers in the 1950s discovered that chloroplasts and mitochondria contain their own genetic material. The issue of endosymbiosis was hotly debated after Lynn Margulis published a book entitled *Origin of Eukaryotic Cells* (1970). During the 1970s and 1980s, the advent of molecular genetic techniques allowed researchers to analyze genes from chloroplasts, mitochondria, bacteria, and eukaryotic nuclear genomes. They found that genes in chloroplasts and mitochondria are very similar to bacterial genes but not as similar to those found within the nucleus of eukaryotic cells. This observation provided strong support for the endosymbiotic origin of mitochondria and chloroplasts, which is now widely accepted.

The endosymbiosis theory proposes that the relationship provided eukaryotic cells with useful cellular characteristics. Chloroplasts were derived from cyanobacteria, a bacterial species that is capable of photosynthesis. The ability to carry out photosynthesis enabled algal and plant cells to use the energy from sunlight. By comparison, mitochondria are thought to have been derived from a different type of bacteria known as gram-negative nonsulfur purple bacteria. In this case, the endosymbiotic relationship enabled eukaryotic cells to synthesize greater amounts of ATP. It is less clear how the relationship would have been beneficial to cyanobacteria or purple bacteria, though the cytosol of a eukaryotic cell may have provided a stable environment with an adequate supply of nutrients.

During the evolution of eukaryotic species, most genes that were originally found in the genome of the primordial cyanobacteria and purple bacteria have been lost or transferred from the organelles to the nucleus. The sequences of certain genes within the nucleus are consistent with their origin within an organelle. Such genes are more similar in their DNA sequence to known bacterial genes than to their eukaryotic counterparts. Therefore, researchers have concluded that these genes have been removed from the mitochondrial and chloroplast chromosomes and relocated to the nuclear chromosomes. This has occurred many times throughout evolution, so modern mitochondria and chloroplasts have lost most of the genes that are still found in present-day purple bacteria and cyanobacteria.

Most of this gene transfer occurred early in mitochondrial and chloroplast evolution. The functional transfer of mitochondrial genes seems to have ceased in animals, but gene transfer from mitochondria and chloroplasts to the nucleus continues to occur in plants at a low rate. The molecular mechanism of gene transfer is not entirely understood, but the direction of transfer is well established. During evolution, gene transfer has occurred primarily from the organelles to the nucleus. For example, about 1500 genes have been transferred from the mitochondrial genome to the nuclear genome of eukaryotes. Transfer of genes from the nucleus to the organelles has almost never occurred, although one example is known of a nuclear gene in plants that has been transferred to the mitochondrial genome. This unidirectional gene transfer from organelles to the nucleus partly explains why the organellar genomes now contain relatively few genes. In addition, gene transfer can occur between organelles. It can happen between two mitochondria, between two chloroplasts, and between a chloroplast and mitochondrion. Overall, the transfer of genetic material between the nucleus, chloroplasts, and mitochondria is an established phenomenon, although its biological benefits remain unclear.

Eukaryotic Cells Occasionally Contain Symbiotic Infective Particles

Other unusual endosymbiotic relationships have been identified in eukaryotic organisms. Several examples are known in which infectious particles establish a symbiotic relationship with their host. In some cases, research indicates that symbiotic infectious particles are bacteria that exist within the cytoplasm of eukaryotic cells. Although symbiotic infectious particles are relatively uncommon, they have provided interesting and even bizarre examples of the extranuclear inheritance of traits.

In the 1940s, Tracy Sonneborn studied a phenomenon known as the killer trait in the protozoan *Paramecium aurelia*. Killer paramecia secrete a substance called paramecin, which kills some but not all strains of paramecia. Sonneborn found that killer strains contain particles in their cytoplasm known as kappa particles. Each kappa particle is 0.4 μm long and has its own DNA. Genes within the kappa particle encode the paramecin toxin. In addition, other kappa particle genes provide the killer paramecia with resistance to the toxin.

Nonkiller paramecia are killed when mixed with killer paramecia. However, Sonnenborn found that when nonkiller paramecia were mixed with a cell extract derived from killer paramecia, the kappa particles within the extract are taken up by the nonkiller strains and convert them into killer strains. In other words, the extranuclear particle that determines the killer trait is infectious.

Infectious particles have also been identified in fruit flies. Philippe l'Heritier identified strains of *Drosophila melanogaster* that are highly sensitive to killing by CO_2. Reciprocal crosses between CO_2-sensitive and normal flies revealed that the trait is inherited in a non-Mendelian manner. Furthermore, cell extracts from a sensitive fly can infect a normal fly and make it sensitive to CO_2.

Another example of an infectious particle in fruit flies involves a trait known as sex ratio in which affected flies produce progenies with a large excess of females. Chana Malogolowkin and Donald Poulson discovered one strain of *Drosophila willistoni* in which most of the offspring of female flies were daughters; nearly all the male offspring died. The sex ratio trait is transmitted from mother to offspring. The rare surviving males do not transmit this trait to their male or female offspring. This result indicates a maternal inheritance pattern for the sex ratio trait. The agent in the cytoplasm of female flies responsible for the sex ratio trait was later found to be a symbiotic bacterium, which was named *Spiroplasma poulsonii*. Its presence is usually lethal to males but not to females. This infective agent can be extracted from the tissues of adult females and used to infect the females of a normal strain of flies.

KEY TERMS

Page 100. maternal effect, nuclear genes
Page 101. reciprocal cross
Page 103. epigenetic inheritance, dosage compensation, X inactivation
Page 104. Barr body, Lyon hypothesis
Page 106. clone
Page 108. X-inactivation center (Xic), X chromosomal controlling element (Xce)
Page 109. genomic imprinting, monoallelic expression
Page 111. DNA methylation, imprinting control region (ICR)
Page 113. extranuclear inheritance, cytoplasmic inheritance
Page 114. nucleoid, mitochondrial DNA (mtDNA)
Page 115. chloroplast DNA (cpDNA)
Page 116. maternal inheritance, heteroplasmy
Page 117. petites
Page 118. heterogamous, paternal leakage
Page 119. endosymbiosis, endosymbiosis theory

CHAPTER SUMMARY

- Non-Mendelian inheritance refers to inheritance patterns that cannot be easily explained by Mendel's experiments.

5.1 Maternal Effect

- Maternal effect is an inheritance pattern in which the genotype of the mother determines the phenotype of the offspring. It occurs because gene products of maternal effect genes are transferred from nurse cells to the oocyte. These gene products affect early stages of development (see Figures 5.1, 5.2).

5.2 Epigenetic Inheritance

- Epigenetic inheritance is a pattern in which a gene or chromosome is modified and gene expression is altered, but the modification is not permanent over the course of many generations.
- Dosage compensation often occurs in species that differ in their sex chromosomes (see Table 5.1).
- In mammals, the process of X inactivation in females compensates for the single X chromosome found in males. The inactivated X chromosome is called a Barr body. The process can lead to a variegated phenotype, such as a calico cat (see Figures 5.3, 5.4).
- After it occurs during embryonic development, the pattern of X inactivation is maintained when cells divide (see Figures 5.5, 5.6).
- X inactivation is controlled by the X-inactivation center that contains the *Xist* and *Tsix* genes. X inactivation occurs as initiation, spreading, and maintenance phases (see Figure 5.7, 5.8).
- Genomic imprinting refers to a marking process in which an offspring expresses a gene that is inherited from one parent but not both (see Figures 5.9, 5.10).
- DNA methylation at imprinting control regions is the marking process that causes imprinting (see Figures 5.11, 5.12).
- Human diseases such as Prader-Willi syndrome and Angelman syndrome are associated with genomic imprinting (see Figure 5.13, Table 5.2).

5.3 Extranuclear Inheritance

- Extranuclear inheritance involves the inheritance of genes that are found in mitochondria or chloroplasts.
- Mitochondria and chloroplasts carry circular chromosomes in a nucleoid region. These circular chromosomes contain relatively few genes compared with the number in the cell nucleus (see Figures 5.14–5.16, Table 5.3).
- Maternal inheritance occurs when organelles, such as mitochondria or chloroplasts, are transmitted via the egg (see Figure 5.17).
- Heteroplasmy for chloroplasts can result in a variegated phenotype (see Figure 5.18).
- Neutral and suppressive petites in yeast are due to defects in mitochondrial DNA and show a non-Mendelian inheritance pattern (see Figure 5.19).
- In the alga *Chlamydomonas*, chloroplasts are transmitted from the mt^+ parent (see Figure 5.20).
- The transmission patterns of mitochondria and chloroplasts vary among different species (see Table 5.4).
- Many diseases are caused by mutations in mitochondrial DNA (see Table 5.5).
- Mitochondria and chloroplasts were derived from an ancient endosymbiotic relationship (see Figure 5.21).
- On rare occasions, eukaryotic cells may contain infectious particles.

PROBLEM SETS & INSIGHTS

Solved Problems

S1. Our understanding of maternal effect genes has been greatly aided by their identification in experimental organisms such as *Drosophila melanogaster* and *Caenorhabditis elegans*. In experimental organisms with a short generation time, geneticists have successfully searched for mutant alleles that prevent the normal process of embryonic development. In many cases, the offspring die at early embryonic or larval stages. These are called maternal effect lethal alleles. How would a researcher identify a mutation that produced a recessive maternal effect lethal allele?

Answer: A maternal effect lethal allele can be identified when a phenotypically normal mother produces only offspring with gross developmental abnormalities. For example, let's call the normal allele *N* and the maternal effect lethal allele *n*. A cross between two flies that are heterozygous for a maternal effect lethal allele would produce 1/4 of the offspring with a homozygous genotype, *nn*. These flies are viable because of the maternal effect. Their mother would be *Nn* and provide the *n* egg with a sufficient amount of *N* gene product so that the *nn* flies would develop properly. However, homozygous *nn* females cannot provide their eggs with any normal gene product. Therefore, all of their offspring are abnormal and die during early stages.

S2. A maternal effect gene in *Drosophila*, called *torso*, is found as a recessive allele that prevents the correct development of anterior- and posterior-most structures. A wild-type male is crossed to a female of unknown genotype. This mating produces 100% larva that are missing their anterior- and posterior-most structures and therefore die during early development. What is the genotype and phenotype of the female fly in this cross? What are the genotypes and phenotypes of the female fly's parents?

Answer: Because this cross produces 100% abnormal offspring, the female fly must be homozygous for the abnormal *torso* allele. Even so, the female fly must be phenotypically normal in order to reproduce. This female fly had a mother that was heterozygous for a normal and abnormal *torso* allele and a father that was either heterozygous or homozygous for the abnormal *torso* allele.

$torso^+\ torso^-$ (grandmother) $\times$ $torso^+\ torso^-$ or $torso^-\ torso^-$ (grandfather)

$\downarrow$

$torso^-\ torso^-$ (mother of 100% abnormal offspring)

This female fly is phenotypically normal because its mother was heterozygous and provided the gene products of the $torso^+$ allele from the nurse cells. However, this homozygous female will produce only abnormal offspring because it cannot provide them with the normal $torso^+$ gene products.

S3. An individual with Angelman syndrome produced an offspring with Prader-Willi syndrome. Why does this occur? What are the sexes of the parent with Angelman syndrome and the offspring with Prader-Willi syndrome?

Answer: These two different syndromes are most commonly caused by a small deletion in chromosome 15. In addition, genomic imprinting plays a role because genes in this deleted region are differentially imprinted, depending on sex. If this deletion is inherited from the paternal parent, the offspring develops Prader-Willi syndrome. Therefore, in this problem, the person with Angelman syndrome must have been a male because he produced a child with Prader-Willi syndrome. The child could be either a male or female.

S4. In yeast, a haploid petite mutant also carries a mutant gene that requires the amino acid histidine for growth. The petite his^- strain is crossed to a wild-type his^+ strain to yield the following tetrad:

2 cells: petite his^-

2 cells: petite his^+

Explain the inheritance of the petite and his^- mutations.

Answer: The his^- and his^+ alleles are segregating in a 2:2 ratio. This result indicates a nuclear pattern of inheritance. By comparison, all four cells in this tetrad have a petite phenotype. This is a suppressive petite that arises from a mitochondrial mutation.

S5. Suppose that you are a horticulturist who has recently identified an interesting plant with variegated leaves. How would you determine if this trait is nuclearly or cytoplasmically inherited?

Answer: Make crosses and reciprocal crosses involving normal and variegated strains. In many species, chloroplast genomes are inherited maternally, although this is not always the case. In addition, a significant percentage of paternal leakage may occur. Nevertheless, when reciprocal crosses yield different outcomes, an organellar mode of inheritance is possibly at work.

S6. A phenotype that is similar to a yeast suppressive petite was also identified in the mold *Neurospora crassa*. Mary and Herschel Mitchell identified a slow-growing mutant that they called *poky*. Unlike yeast, which are isogamous (i.e., produce one type of gamete), *Neurospora* is sexually dimorphic and produces male and female reproductive structures. When a *poky* strain of *Neurospora* was crossed to a wild-type strain, the results were different between reciprocal crosses. If a *poky* mutant was the female parent, all spores exhibited the *poky* phenotype. By comparison, if the wild-type strain was the female parent, all spores were wild type. Explain these results.

Answer: These genetic studies indicate that the *poky* mutation is maternally inherited. The cytoplasm of the female reproductive cells provides the offspring with their mitochondria. Besides these genetic studies, the Mitchells and their collaborators showed that *poky* mutants are defective in certain cytochromes, which are iron-containing proteins that are known to be located in the mitochondria.

Conceptual Questions

C1. Define the term epigenetic inheritance, and describe two examples.

C2. Describe the inheritance pattern of maternal effect genes. Explain how the maternal effect occurs at the cellular level. What are the expected functional roles of the proteins that are encoded by maternal effect genes?

C3. A maternal effect gene exists in a dominant *N* (normal) allele and a recessive *n* (abnormal) allele. What would be the ratios of genotypes and phenotypes for the offspring of the following crosses?

A. *nn* female × *NN* male

B. *NN* female × *nn* male

C. *Nn* female × *Nn* male

C4. A *Drosophila* embryo dies during early embryogenesis due to a recessive maternal effect allele called *bicoid*. The wild-type allele is designated $bicoid^+$. What are the genotypes and phenotypes of the embryo's mother and maternal grandparents?

C5. For Mendelian traits, the nuclear genotype (i.e., the alleles found on chromosomes in the cell nucleus) directly influences an offspring's traits. In contrast, for non-Mendelian inheritance patterns, the offspring's phenotype cannot be reliably predicted solely from its genotype. For the following traits, what do you need to know to predict the phenotypic outcome?

A. Dwarfism due to a mutant *Igf2* allele

B. Snail coiling direction

C. Leber hereditary optic neuropathy

C6. Suppose a maternal effect gene exists as a normal dominant allele and an abnormal recessive allele. A mother who is phenotypically abnormal produces all normal offspring. Explain the genotype of the mother.

C7. Suppose that a gene affects the anterior morphology in house flies and is inherited as a maternal effect gene. The gene exists in a normal allele, *H*, and a recessive allele, *h*, which causes a small head. A female fly with a normal head is mated to a true-breeding male with a small head. All of the offspring have small heads. What are the genotypes of the mother and offspring? Explain your answer.

C8. Explain why maternal effect genes exert their effects during the early stages of development.

C9. As described in Chapter 19, researchers have been able to "clone" mammals by fusing a cell having a diploid nucleus (i.e., a somatic cell) with an egg that has had its (haploid) nucleus removed.

A. With regard to maternal effect genes, would the phenotype of such a cloned animal be determined by the animal that donated the egg or by the animal that donated the somatic cell? Explain.

B. Would the cloned animal inherit extranuclear traits from the animal that donated the egg or by the animal that donated the somatic cell? Explain.

C. In what ways would you expect this cloned animal to be similar to or different from the animal that donated the somatic cell? Is it fair to call such an animal a "clone" of the animal that donated the diploid nucleus?

C10. With regard to the numbers of sex chromosomes, explain why dosage compensation is necessary.

C11. What is a Barr body? How is its structure different from that of other chromosomes in the cell? How does the structure of a Barr body affect the level of X-linked gene expression?

C12. Among different species, describe three distinct strategies for accomplishing dosage compensation.

C13. Describe when X inactivation occurs and how this leads to phenotypic results at the organism level. In your answer, you should explain why X inactivation causes results such as variegated coat patterns in mammals. Why do two different calico cats have their patches of orange and black fur in different places? Explain whether or not a variegated coat pattern due to X inactivation could occur in marsupials.

C14. Describe the molecular process of X inactivation. This description should include the three phases of inactivation and the role of the Xic. Explain what happens to X chromosomes during embryogenesis, in adult somatic cells, and during oogenesis.

C15. On rare occasions, an abnormal human male is born who is somewhat feminized compared with normal males. Microscopic examination of the cells of one such individual revealed that he has a single Barr body in each cell. What is the chromosomal composition of this individual?

C16. How many Barr bodies would you expect to find in humans with the following abnormal compositions of sex chromosomes?

A. XXY

B. XYY

C. XXX

D. X0 (a person with just a single X chromosome)

C17. Certain forms of human color blindness are inherited as X-linked recessive traits. Hemizygous males are color-blind, but heterozygous females are not. However, heterozygous females sometimes have partial color blindness.

A. Discuss why heterozygous females may have partial color blindness.

B. Doctors identified an unusual case in which a heterozygous female was color-blind in her right eye but had normal color vision in her left eye. Explain how this might have occurred.

C18. A black female cat (X^BX^B) and an orange male cat (X^0Y) were mated to each other and produced a male cat that was calico. Which sex chromosomes did this male offspring inherit from its mother and father? Remember that the presence of the Y chromosome determines maleness in mammals.

C19. What is the spreading phase of X inactivation? Why do you think it is called a spreading phase? Discuss the role of the *Xist* gene in the spreading phase of X inactivation.

C20. When does the erasure and reestablishment phase of genomic imprinting occur? Explain why it is necessary to erase an imprint and then reestablish it in order to always maintain imprinting from the same sex of parent.

C21. In what types of cells would you expect *de novo* methylation to occur? In what cell types would it not occur?

C22. On rare occasions, people are born with a condition known as uniparental disomy. It happens when an individual inherits both copies of a chromosome from one parent and no copies from the other parent. This occurs when two abnormal gametes happen to complement each other to produce a diploid zygote. For example, an abnormal sperm that lacks chromosome 15 could fertilize an egg that contains two copies of chromosome 15. In this situation, the individual would be said to have maternal uniparental disomy 15 because both copies of chromosome 15 were inherited from the mother. Alternatively, an abnormal sperm with two copies of chromosome 15 could fertilize an egg with no copies. This is known as paternal uniparental disomy 15. If a female is born with paternal uniparental disomy 15, would you expect her to be phenotypically normal, have Angelman syndrome (AS), or have Prader-Willi syndrome (PWS)? Explain. Would you expect her to produce normal offspring or offspring affected with AS or PWS?

C23. Genes that cause Prader-Willi syndrome and Angelman syndrome are closely linked along chromosome 15. Although people with these syndromes do not usually reproduce, let's suppose that a couple produces two children with Angelman syndrome. The oldest child (named Pat) grows up and has two children with Prader-Willi syndrome. The second child (named Robin) grows up and has one child with Angelman syndrome.

A. Are Pat and Robin's parents both normal or does one of them have Angelman or Prader-Willi syndrome? If one of them has a disorder, explain why it is the mother or the father.

B. What are the sexes of Pat and Robin? Explain.

C24. How is the process of X inactivation similar to genomic imprinting? How is it different?

C25. What is extranuclear inheritance? Describe three examples.

C26. What is a reciprocal cross? Suppose that a gene is found as a wild-type allele and a recessive mutant allele. What would be the expected outcomes of reciprocal crosses if a true-breeding normal individual was crossed to a true-breeding individual carrying the mutant allele? What would be the results if the gene is maternally inherited?

C27. Among different species, does extranuclear inheritance always follow a maternal inheritance pattern? Why or why not?

C28. What is the phenotype of a petite mutant? Where can a petite mutation occur: in nuclear genes, extranuclear genetic material, or both? What is the difference between a neutral and suppressive petite?

C29. Extranuclear inheritance often correlates with maternal inheritance. Even so, paternal leakage is not uncommon. What is paternal leakage? If a cross produced 200 offspring and the rate of mitochondrial paternal leakage was 3%, how many offspring would be expected to contain paternal mitochondria?

C30. Discuss the structure and organization of the mitochondrial and chloroplast genomes. How large are they, how many genes do they contain, and how many copies of the genome are found in each organelle?

C31. Explain the likely evolutionary origin of mitochondrial and chloroplast genomes. How have the sizes of the mitochondrial and chloroplast genomes changed since their origin? How has this occurred?

C32. Which of the following traits or diseases are determined by nuclear genes?

A. Snail coiling pattern

B. Prader-Willi syndrome

C. Streptomycin resistance in *Chlamydomonas*

D. Leber hereditary optic neuropathy

C33. Acute murine leukemia virus (AMLV) causes leukemia in mice. This virus is easily passed from mother to offspring through the mother's milk. (Note: Even though newborn offspring acquire the virus, they may not develop leukemia until much later in life. Testing can determine if an animal carries the virus.) Describe how the formation of leukemia via AMLV resembles a maternal inheritance pattern. How could you determine that this form of leukemia is not caused by extranuclear inheritance?

C34. Describe how a biparental pattern of extranuclear inheritance would resemble a Mendelian pattern of inheritance for a particular gene. How would they differ?

C35. According to the endosymbiosis theory, mitochondria and chloroplasts are derived from bacteria that took up residence within eukaryotic cells. However, at one time, prior to being taken up by eukaryotic cells, these bacteria were free-living organisms. However, we cannot take a mitochondrion or chloroplast out of a living eukaryotic cell and get it to survive and replicate on its own. Why not?

Experimental Questions

E1. Figure 5.1 describes an example of a maternal effect gene. Explain how Sturtevant deduced a maternal effect gene based on the F_2 and F_3 generations.

E2. Discuss the types of experimental observations that Mary Lyon brought together in proposing her hypothesis concerning X inactivation. In your own words, explain how these observations were consistent with her hypothesis.

E3. Chapter 18 describes three blotting methods (i.e., Southern blotting, Northern blotting, and Western blotting) that are used to detect specific genes and gene products. Southern blotting detects DNA, Northern blotting detects RNA, and Western blotting detects proteins. Suppose that a female fruit fly is heterozygous for a maternal effect gene, which we will call gene *B*. The female is *Bb*. The normal allele, *B*, encodes a functional mRNA that is 550 nucleotides long. A recessive allele, *b*, encodes a shorter mRNA that is 375 nucleotides long. (Allele *b* is due to a deletion within this gene.) How could you use one or more of these techniques to show that nurse cells transfer gene products from gene *B* to developing eggs? You may assume that you can dissect the ovaries of fruit flies and isolate eggs separately from nurse cells. In your answer, describe your expected results.

E4. As a hypothetical example, a trait in mice results in mice with very long tails. You initially have a true-breeding strain with normal tails and a true-breeding strain with long tails. You then make the following types of crosses:

Cross 1: When true-breeding females with normal tails are crossed to true-breeding males with long tails, all F_1 offspring have long tails.

Cross 2: When true-breeding females with long tails are crossed to true-breeding males with normal tails, all F_1 offspring have normal tails.

Cross 3: When F_1 females from cross 1 are crossed to true-breeding males with normal tails, all offspring have normal tails.

Cross 4: When F_1 males from cross 1 are crossed to true-breeding females with long tails, half of the offspring have normal tails and half have long tails.

Explain the pattern of inheritance of this trait.

E5. You have a female snail that coils to the right, but you do not know its genotype. You may assume that right coiling (*D*) is dominant to left coiling (*d*). You also have male snails at your disposal of known genotype. How would you determine the genotype of this female snail? In your answer, describe your expected results depending on whether the female is *DD*, *Dd*, or *dd*.

E6. On a recent camping trip, you find one male snail on a deserted island that coils to the right. However, in this same area, you find several shells (not containing living snails) that coil to the left. Therefore, you conclude that you are not certain of the genotype of this male snail. On a different island, you find a large colony of snails of the same species. All of these snails coil to the right, and every snail shell that you find on this second island coils to the right. With regard to the maternal effect gene that determines coiling pattern, how would you determine the genotype of the male snail that you found on the deserted island? In your answer, describe your expected results.

E7. Figure 5.6 describes the results of X inactivation in mammals. If fast and slow alleles of glucose-6-phosphate dehydrogenase (*G-6-PD*) exist in other species, what would be the expected results of gel electrophoresis for a heterozygous female of the following species?

A. Marsupial

B. *Drosophila melanogaster*

C. *Caenorhabditis elegans* (Note: We are considering the hermaphrodite in *C. elegans* to be equivalent to a female.)

E8. Two male mice, which we will call male A and male B, are both phenotypically normal. Male A was from a litter that contained half phenotypically normal mice and half dwarf mice. The mother of male A was known to be homozygous for the normal *Igf2* allele. Male B was from a litter of eight mice that were all phenotypically normal. The parents of male B were a phenotypically normal male and a dwarf female. Male A and male B were put into a cage with two female mice that we will call female A and female B. Female A is dwarf, and female B is phenotypically normal. The parents of these two females were unknown, although it was known that they were from the same litter. The mice were allowed to mate with each other, and the following data were obtained:

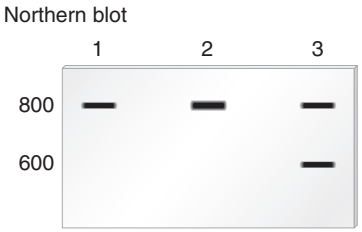
Female A gave birth to three dwarf babies and four normal babies.

Female B gave birth to four normal babies and two dwarf babies.

Which male(s) mated with female A and female B? Explain.

E9. In the experiment of Figure 5.6, why does a clone of cells produce only one type of G-6-PD enzyme? What would you expect to happen if a clone was derived from an early embryonic cell? Why does the initial sample of tissue produce both forms of G-6-PD?

E10. Chapter 18 describes a blotting method known as Northern blotting that can be used to determine the amount of mRNA produced by a particular gene. In this method, the amount of a specific mRNA produced by cells is detected as a band on a gel. If one type of cell produces twice as much of a particular mRNA as another cell, the band will appear twice as dark. Also, sometimes mutations affect the length of mRNA that is transcribed from a gene. For example, a small deletion within a gene may shorten an mRNA. Northern blotting also can discern the sizes of mRNAs.



Lane 1 is a Northern blot of mRNA from cell type A that is 800 nucleotides long.

Lane 2 is a Northern blot of the same mRNA from cell type B. (Cell type B produces twice as much of this RNA as cell type A.)

Lane 3 shows a heterozygote in which one of the two genes has a deletion, which shortens the mRNA by 200 nucleotides.

Here is the question. Suppose an X-linked gene exists as two alleles: *B* and *b*. Allele *B* encodes an mRNA that is 750 nucleotides long, and allele *b* encodes a shorter mRNA that is 675 nucleotides long. Draw the expected results of a Northern blot using mRNA isolated from the same type of somatic cells taken from the following individuals:

A. First lane is mRNA from an X^bY male fruit fly.

Second lane is mRNA from an X^bX^b female fruit fly.

Third lane is mRNA from an X^BX^b female fruit fly.

B. First lane is mRNA from an X^BY male mouse.

Second lane is mRNA from an X^BX^b female mouse.*

Third lane is mRNA from an X^BX^B female mouse.*

*The sample is taken from an adult female mouse. It is not a clone of cells. It is a tissue sample, like the one described in the experiment of Figure 5.6.

C. First lane is mRNA from an X^B0 male *C. elegans*.

Second lane is mRNA from an X^BX^b hermaphrodite *C. elegans*.

Third lane is mRNA from an X^BX^B hermaphrodite *C. elegans*.

E11. A variegated trait in plants is analyzed using reciprocal crosses. The following results are obtained:

Variegated female × Normal male	Normal female × Variegated male
↓	↓
1024 variegated + 52 normal	1113 normal + 61 variegated

Explain this pattern of inheritance.

E12. Ruth Sager and her colleagues discovered that the mode of inheritance of streptomycin resistance in *Chlamydomonas* could be altered if the mt^+ cells were exposed to UV irradiation prior to mating. This exposure dramatically increased the frequency of biparental inheritance. What would be the expected outcome of a cross between an mt^+ sm^r and an mt^- sm^s strain in the absence of UV irradiation? How would the result differ if the mt^+ strain was exposed to UV light?

E13. Take a look at Figure 5.19 and describe how you could experimentally distinguish between yeast strains that are neutral petites and those that are suppressive petites.

Questions for Student Discussion/Collaboration

1. Recessive maternal effect genes are identified in flies (for example) when a phenotypically normal mother cannot produce any normal offspring. Because all of the offspring are dead, this female fly cannot be used to produce a strain of heterozygous flies that could be used in future studies. How would you identify heterozygous individuals that are carrying a recessive maternal effect allele? How would you maintain this strain of flies in a laboratory over many generations?

2. What is an infective particle? Discuss the similarities and differences between infective particles and organelles such as mitochondria and chloroplasts. Do you think the existence of infective particles supports the endosymbiosis theory of the origin of mitochondria and chloroplasts?

Note: All answers appear at the Website for this textbook; the answers to even-numbered questions are in the back of the textbook.