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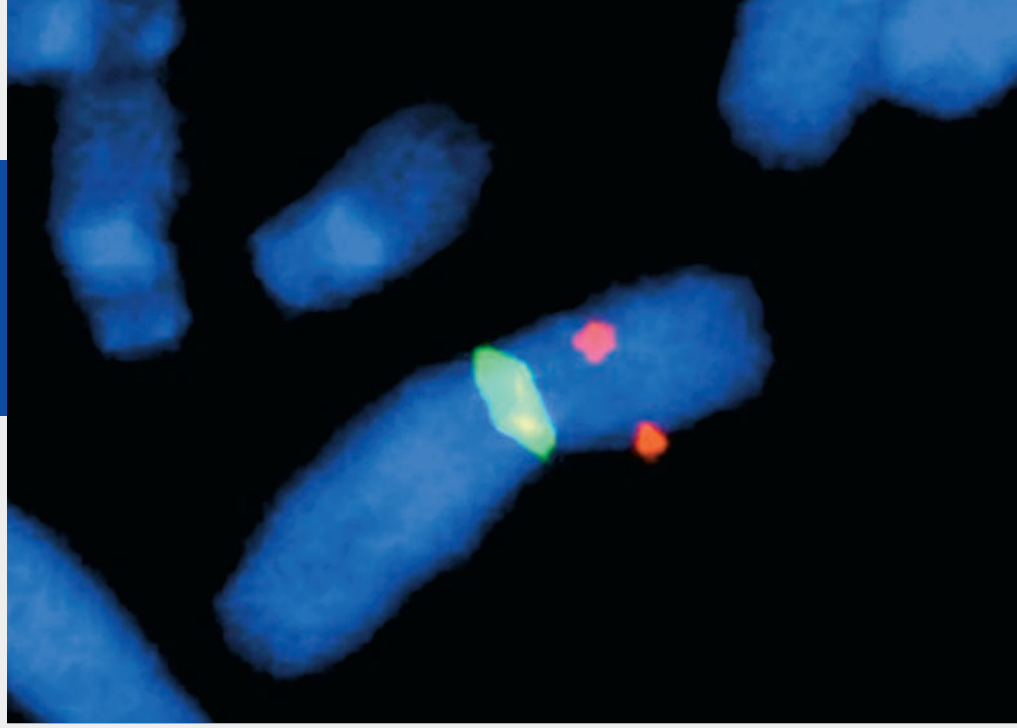


Sex Determination and Sex Chromosomes

A human X chromosome highlighted using fluorescence *in situ* hybridization (FISH), a method in which specific probes bind to specific sequences of DNA. The green fluorescence probe binds to DNA at the centromere of X chromosomes. The red fluorescence probe binds to the DNA sequence of the X-linked Duchenne muscular dystrophy (DMD) gene.

CHAPTER CONCEPTS

- A variety of mechanisms have evolved that result in sexual differentiation, leading to sexual dimorphism and greatly enhancing the production of genetic variation within species.
- Often, specific genes, usually on a single chromosome, cause maleness or femaleness during development.
- In humans, the presence of extra X or Y chromosomes beyond the diploid number may be tolerated but often leads to syndromes demonstrating distinctive phenotypes.
- While segregation of sex-determining chromosomes should theoretically lead to a one-to-one sex ratio of males to females, in humans the actual ratio favors males at conception.
- In mammals, females inherit two X chromosomes compared to one in males, but the extra genetic information in females is compensated for by random inactivation of one of the X chromosomes early in development.
- In some reptilian species, temperature during incubation of eggs determines the sex of offspring.

In the biological world, a wide range of reproductive modes and life cycles are observed. Some organisms are entirely asexual, displaying no evidence of sexual reproduction. Other organisms alternate between short periods of sexual reproduction and prolonged periods of asexual reproduction. In most diploid eukaryotes, however, sexual reproduction is the only natural mechanism for producing new members of the species. The perpetuation of all sexually reproducing organisms depends ultimately on an efficient union of gametes during fertilization. In turn, successful fertilization depends on some form of **sexual differentiation** in the reproductive organisms. Even though it is not overtly evident, this differentiation occurs in organisms as low on the evolutionary scale as bacteria and single-celled eukaryotic algae. In more complex forms of life, the differentiation of the sexes is more evident as phenotypic dimorphism of males and females. The ancient symbol for iron and for Mars, depicting a shield and spear (♂), and the ancient symbol for copper and for Venus, depicting a mirror (♀), have also come to symbolize maleness and femaleness, respectively.

Dissimilar, or **heteromorphic**, **chromosomes**, such as the XY pair in mammals, characterize one sex or the other in a wide range of species, resulting in their label as **sex chromosomes.** Nevertheless, it is genes, rather than chromosomes, that ultimately serve as the underlying basis of **sex determination**. As we will see, some of these genes are present on sex chromosomes, but others are autosomal. Extensive investigation has revealed a wide variation in sex-chromosome systems—even in closely related organisms—suggesting that mechanisms controlling sex

determination have undergone rapid evolution many times in the history of life.

In this chapter, we delve more deeply into what is known about the genetic basis for the determination of sexual differences, with a particular emphasis on two organisms: our own species, representative of mammals; and *Drosophila*, on which pioneering sex-determining studies were performed.

7.1 X and Y Chromosomes Were First Linked to Sex Determination Early in the Twentieth Century

How sex is determined has long intrigued geneticists. In 1891, Hermann Henking identified a nuclear structure in the sperm of certain insects, which he labeled the X-body. Several years later, Clarence McClung showed that some of the sperm in grasshoppers contain an unusual genetic structure, called a *heterochromosome,* but the remainder of the sperm lack this structure. He mistakenly associated the presence of the heterochromosome with the production of male progeny. In 1906, Edmund B. Wilson clarified Henking and McClung's findings when he demonstrated that female somatic cells in the butterfly *Protenor* contain 14 chromosomes, including 12 autosomes (A) and two X chromosomes. During oogenesis, an even reduction occurs, producing gametes with seven chromosomes, including one X chromosome (6A + X). Male somatic cells, on the other hand, contain only 13 chromosomes, including one X chromosome. During spermatogenesis, gametes are produced containing either six chromosomes, without an X (6A), or seven chromosomes, one of which is an X (6A + X). Fertilization by X-bearing sperm results in female offspring, and fertilization by X-deficient sperm results in male offspring [**Figure 7.1(a)**].

The presence or absence of the X chromosome in male gametes provides an efficient mechanism for sex determination in this species and also produces a 1:1 sex ratio in the resulting offspring.

Wilson also experimented with the milkweed bug *Lygaeus turcicus*, in which both sexes have 14 chromosomes. Twelve of these are autosomes. In addition, the females have two X chromosomes, while the males have only a single X and a smaller heterochromosome labeled the **Y chromosome.** Females in this species produce only gametes of the (6A + X) constitution, but males produce two types of gametes in equal proportions, (6A + X) and (6A + Y). Therefore, following random fertilization, equal numbers of male and female progeny will be produced with distinct chromosome complements [**Figure 7.1(b)**].

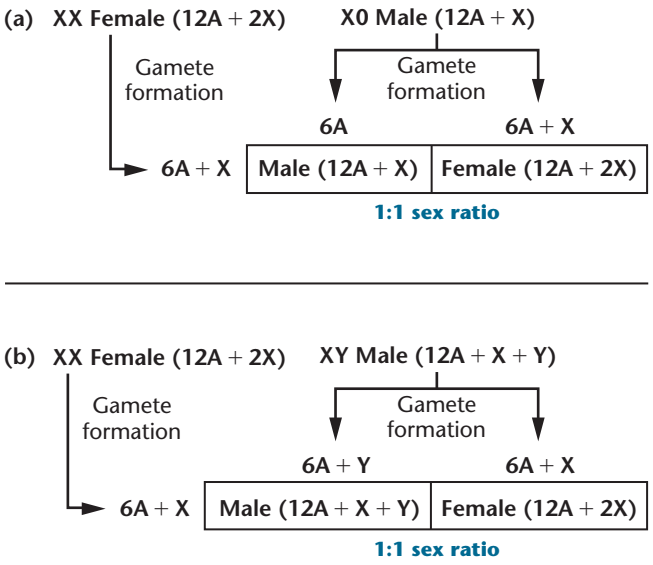


FIGURE 7.1 (a) Sex determination where the heterogametic sex (the male in this example) is X0 and produces gametes with or without the X chromosome; (b) sex determination, where the heterogametic sex (again, the male in this example) is XY and produces gametes with either an X or a Y chromosome. In both cases, the chromosome composition of the offspring determines its sex.

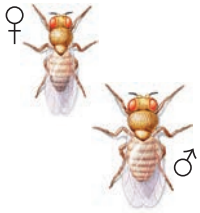
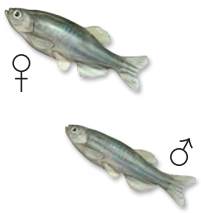
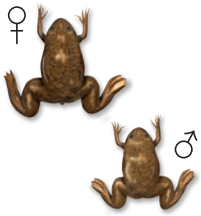
In *Protenor* and *Lygaeus*, males produce gametes with different chromosome compositions. As a result, they are described as the **heterogametic sex,** and in effect, their gametes ultimately determine the sex of the progeny in those species. In such cases, the female, which has like sex chromosomes, is the **homogametic sex,** producing uniform gametes with regard to chromosome numbers and types.

The male is not always the heterogametic sex. In some organisms, the female produces unlike gametes, exhibiting either the *Protenor* XX/XO or *Lygaeus* XX/XY mode of sex determination. Examples include certain moths and butterflies, some fish, reptiles, amphibians, at least one species of plants (*Fragaria orientalis*), and most birds. To immediately distinguish situations in which the female is the heterogametic sex, some geneticists use the notation **ZZ/ZW,** where ZZ is the homogametic male and ZW is the heterogametic female, instead of the XX/XY notation. For example, chickens are so denoted. The sex chromosome composition for popular model organisms in genetics is shown in Table 7.1.

7.2 The Y Chromosome Determines Maleness in Humans

The first attempt to understand sex determination in our own species occurred almost 100 years ago and involved the visual examination of chromosomes in dividing cells.

TABLE 7.1 Sex Chromosome Compositions of Common Model Organisms

	Caenorhabditis elegans	*Drosophila melanogaster*	*Mus musculus*	*Danio rerio*	*Xenopus laevis*
Model Organism					
Sex Chromosomes	XX XO	XX XY	XX XY	None	ZW ZZ

Efforts were made to accurately determine the diploid chromosome number of humans, but because of the relatively large number of chromosomes, this proved to be quite difficult. Then, in 1956, Joe Hin Tjio and Albert Levan discovered an effective way to prepare chromosomes for accurate viewing. This technique led to a strikingly clear demonstration of metaphase stages showing that 46 was indeed the human diploid number. Later that same year, C. E. Ford and John L. Hamerton, also working with testicular tissue, confirmed this finding. The familiar karyotypes of a human male (Figure 2.4) illustrate the difference in size between the human X and Y chromosomes.

Of the normal 23 pairs of human chromosomes, one pair was shown to vary in configuration in males and females. These two chromosomes were designated the X and Y sex chromosomes. The human female has two X chromosomes, and the human male has one X and one Y chromosome.

We might believe that this observation is sufficient to conclude that the Y chromosome determines maleness. However, several other interpretations are possible. The Y could play no role in sex determination; the presence of two X chromosomes could cause femaleness; or maleness could result from the lack of a second X chromosome. The evidence that clarified which explanation was correct came from study of the effects of human sex-chromosome variations, described in the following section. As such investigations revealed, the Y chromosome does indeed determine maleness in humans.

Klinefelter and Turner Syndromes

Around 1940, scientists identified two human abnormalities characterized by aberrant sexual development, **Klinefelter syndrome (47,XXY)** and **Turner syndrome (45,X).*** Individuals with Klinefelter syndrome are generally tall and have long arms and legs and large hands and feet. They usually have genitalia and internal ducts that are male, but their testes are rudimentary and fail to produce sperm. At the same time, feminine sexual development is not entirely suppressed. Slight enlargement of the breasts (gynecomastia) is common, and the hips are often rounded. This ambiguous sexual development, referred to as intersexuality, can lead to abnormal social development. Intelligence is often below the normal range as well.

In Turner syndrome, the affected individual has female external genitalia and internal ducts, but the ovaries are rudimentary. Other characteristic abnormalities include short stature (usually under 5 feet), skin flaps on the back of the neck, and underdeveloped breasts. A broad, shieldlike chest is sometimes noted. Intelligence is usually normal.

In 1959, the karyotypes of individuals with these syndromes were determined to be abnormal with respect to the sex chromosomes. Individuals with Klinefelter syndrome have more than one X chromosome. Most often they have an XXY complement in addition to 44 autosomes [**Figure 7.2(a)**], which is why people with this karyotype are designated 47,XXY. Individuals with Turner syndrome most often have only 45 chromosomes, including just a single X chromosome; thus, they are designated 45,X [**Figure 7.2(b)**]. Note the convention used in designating these chromosome compositions. The number states the total number of chromosomes present, and the information after the comma indicates the deviation from the normal diploid content. Both conditions result from **nondisjunction,** the failure of the sex chromosomes to segregate properly during meiosis (nondisjunction is described in Chapter 8 and illustrated in Figure 8.1).

* Although the possessive form of the names of eponymous syndromes is sometimes used (e.g., Klinefelter's syndrome), the current preference is to use the nonpossessive form.

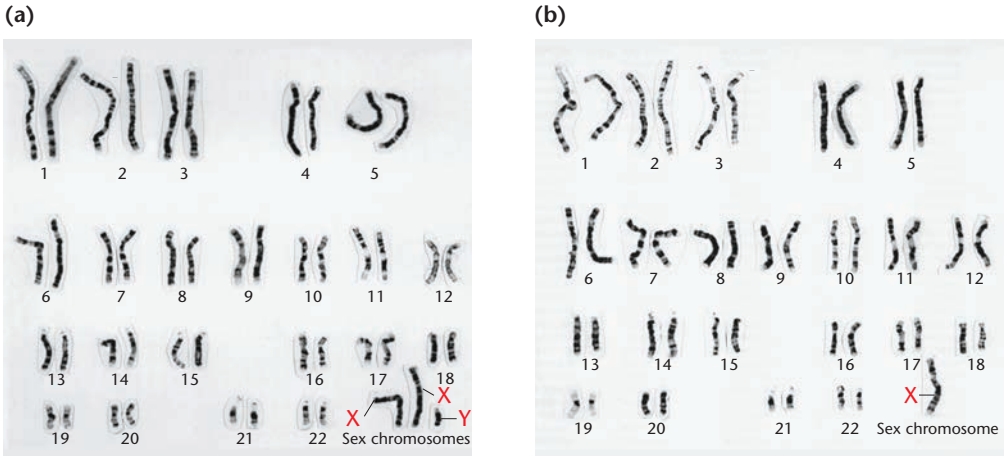


FIGURE 7.2 The karyotypes of individuals with (a) Klinefelter syndrome (47,XXY) and (b) Turner syndrome (45,X).

These Klinefelter and Turner karyotypes and their corresponding sexual phenotypes led scientists to conclude that the Y chromosome determines maleness in humans. In its absence, the person's sex is female, even if only a single X chromosome is present. The presence of the Y chromosome in the individual with Klinefelter syndrome is sufficient to determine maleness, even though male development is not complete. Similarly, in the absence of a Y chromosome, as in the case of individuals with Turner syndrome, no masculinization occurs. Note that we cannot conclude anything regarding sex determination under circumstances where a Y chromosome is present without an X because Y-containing human embryos lacking an X chromosome (designated 45,Y) do not survive.

Klinefelter syndrome occurs in about 1 of every 660 male births and is the most common sex chromosome disorder in males. The karyotypes **48,XXXY, 48,XXYY, 49,XXXXY**, and **49,XXXYY** are similar phenotypically to 47,XXY, but manifestations are often more severe in individuals with a greater number of X chromosomes. Recent studies have also shown that the variability in phenotypes for men with a 47,XXY genotype is correlated with copy number variations (CNVs), particularly duplications, on the X chromosomes.

Turner syndrome can also result from karyotypes other than 45,X, including individuals called **mosaics,** whose somatic cells display two different genetic cell lines, each exhibiting a different karyotype. Such cell lines result from a mitotic error during early development, the most common chromosome combinations being **45,X/46,XY** and **45,X/46,XX.** Thus, an embryo that began life with a normal karyotype can give rise to an individual whose cells show a mixture of karyotypes and who exhibits varying aspects of this syndrome.

Turner syndrome is observed in about 1 in 2000 female births, a frequency much lower than that for Klinefelter syndrome. One explanation for this difference is the observation that a substantial majority of 45,X fetuses die *in utero* and are aborted spontaneously. Thus, a similar frequency of the two syndromes may occur at conception.

47,XXX Syndrome

The abnormal presence of three X chromosomes along with a normal set of autosomes (**47,XXX**) results in female differentiation. The highly variable syndrome that accompanies this genotype, often called **triplo-X,** occurs in about 1 of 1000 female births. Frequently, 47,XXX women are perfectly normal and may remain unaware of their abnormality in chromosome number unless a karyotype is done. In other cases, underdeveloped secondary sex characteristics, sterility, delayed development of language and motor skills, and mental retardation may occur. In rare instances, **48,XXXX** (tetra-X) and **49,XXXXX** (penta-X) karyotypes have been reported. The syndromes associated with these karyotypes are similar to but more pronounced than the 47,XXX syndrome. Thus, in many cases, the presence of additional X chromosomes appears to disrupt the delicate balance of genetic information essential to normal female development.

47,XYY Condition

Another human condition involving the sex chromosomes is **47,XYY.** Studies of this condition, in which the only deviation from diploidy is the presence of an additional Y chromosome in an otherwise normal male karyotype, were initiated in 1965 by Patricia Jacobs. She discovered that 9 of 315 males in a Scottish maximum security prison had the 47,XYY karyotype. These males were significantly above average in height and had been incarcerated as a

result of dangerous, violent, or criminal propensities. Of the nine males studied, seven were of subnormal intelligence, and all suffered personality disorders. Several other studies produced similar findings.

The possible correlation between this chromosome composition and criminal behavior piqued considerable interest, and extensive investigation of the phenotype and frequency of the 47,XYY condition in both criminal and noncriminal populations ensued. Above-average height (usually over 6 feet) and subnormal intelligence were substantiated, and the frequency of males displaying this karyotype was indeed revealed to be higher in penal and mental institutions compared with unincarcerated populations [one study showed 29 XYY males when 28,366 were examined (0.10%)]. A particularly relevant question involves the characteristics displayed by the XYY males who are not incarcerated. The only nearly constant association is that such individuals are over 6 feet tall.

A study to further address this issue was initiated in 1974 to identify 47,XYY individuals at birth and to follow their behavioral patterns during preadult and adult development. While the study was considered unethical and soon abandoned, it has became clear that there are many XYY males present in the population who do not exhibit antisocial behavior and who lead normal lives. Therefore, we must conclude that there is a high, but not constant, correlation between the extra Y chromosome and the predisposition of these males to exhibit behavioral problems.

Sexual Differentiation in Humans

Once researchers had established that, in humans, it is the Y chromosome that houses genetic information necessary for maleness, they attempted to pinpoint a specific gene or genes capable of providing the "signal" responsible for sex determination. Before we delve into this topic, it is useful to consider how sexual differentiation occurs in order to better comprehend how humans develop into sexually dimorphic males and females. During early development, every human embryo undergoes a period when it is potentially hermaphroditic. By the fifth week of gestation, gonadal primordia (the tissues that will form the gonad) arise as a pair of **gonadal (genital) ridges** associated with each embryonic kidney. The embryo is potentially hermaphroditic because at this stage its gonadal phenotype is sexually indifferent—male or female reproductive structures cannot be distinguished, and the gonadal ridge tissue can develop to form male or female gonads. As development progresses, primordial germ cells migrate to these ridges, where an outer cortex and inner medulla form (*cortex* and *medulla* are the outer and inner tissues of an organ, respectively). The cortex is capable of developing into an ovary, while the medulla may develop into a testis. In addition, two sets of undifferentiated ducts called the Wolffian and Müllerian ducts exist in each embryo. Wolffian ducts differentiate into other organs of the male reproductive tract, while Müllerian ducts differentiate into structures of the female reproductive tract.

Because gonadal ridges can form either ovaries or testes, they are commonly referred to as **bipotential gonads.** What switch triggers gonadal ridge development into testes or ovaries? The presence or absence of a Y chromosome is the key. If cells of the ridge have an XY constitution, development of the medulla into a testis is initiated around the seventh week. However, in the absence of the Y chromosome, no male development occurs, the cortex of the ridge subsequently forms ovarian tissue, and the Müllerian duct forms oviducts (Fallopian tubes), uterus, cervix, and portions of the vagina. Depending on which pathway is initiated, parallel development of the appropriate male or female duct system then occurs, and the other duct system degenerates. If testes differentiation is initiated, the embryonic testicular tissue secretes hormones that are essential for continued male sexual differentiation. As we will discuss in the next section, the presence of a Y chromosome and the development of the testes also inhibit formation of female reproductive organs.

In females, as the twelfth week of fetal development approaches, the oogonia within the ovaries begin meiosis, and primary oocytes can be detected. By the twenty-fifth week of gestation, all oocytes become arrested in meiosis and remain dormant until puberty is reached some 10 to 15 years later. In males, on the other hand, primary spermatocytes are not produced until puberty is reached (see Figure 2.11).

As sexual dimorphism is considered, it is important to distinguish between *primary sexual differentiation,* which involves only the gonads, where gametes are produced, and *secondary sexual differentiation,* which involves the overall phenotype of the organism. Secondary effects include clear differences in such organs as mammary glands and external genitalia as well as other traits that differ between males and females.

The Y Chromosome and Male Development

The human Y chromosome, unlike the X, was long thought to be mostly blank genetically. It is now known that this is not true, even though the Y chromosome contains far fewer genes than does the X. Data from the Human Genome Project indicate that the Y chromosome has at least 75 genes, compared to 900–1400 genes on the X. Current analysis of these genes and regions with potential genetic function reveals that some have homologous counterparts on the X chromosome and others do not. In addition, recent work has revealed that a small number of conserved and essential genes previously thought to be lost from the Y chromosome throughout evolution are present on autosomes. Present on both ends of

the Y chromosome are so-called **pseudoautosomal regions (PARs)** that share homology with regions on the X chromosome and synapse and recombine with it during meiosis. The presence of such a pairing region is critical to segregation of the X and Y chromosomes during male gametogenesis. The remainder of the chromosome, about 95 percent of it, does not synapse or recombine with the X chromosome. As a result, it was originally referred to as the *nonrecombining region of the Y (NRY)*. More recently, researchers have designated this region as the **male-specific region of the Y (MSY).** Some portions of the MSY share homology with genes on the X chromosome, and others do not.

The human Y chromosome is diagrammed in **Figure 7.3**. The MSY is divided about equally between *euchromatic* regions, containing functional genes, and *heterochromatic* regions, lacking genes. Within euchromatin, adjacent to the PAR of the short arm of the Y chromosome, is a critical gene that controls male sexual development, called the **sex-determining region Y (SRY)**. In humans, the absence of a Y chromosome almost always leads to female development; thus, this gene is absent from the X chromosome. At six to eight weeks of development, the *SRY* gene becomes active in XY embryos. *SRY* encodes a protein that causes the undifferentiated gonadal tissue of the embryo to form testes. This protein is called the **testis-determining factor (TDF).** *SRY* (or a closely related version) is present in all mammals thus far examined, indicative of its essential function throughout this diverse group of animals.*

Our ability to identify the presence or absence of DNA sequences in rare individuals whose sex-chromosome composition does not correspond to their sexual phenotype has provided evidence that *SRY* is the gene responsible for male sex determination. For example, there are human males who have two X and no Y chromosomes. Often, attached to one of their X chromosomes is the region of the Y that contains *SRY*. There are also females who have one X and one Y chromosome, a condition known as XY sex reversal or Swyer syndrome. Their Y is almost always missing the *SRY* gene or they have a specific mutation in *SRY*. These observations argue strongly in favor of the role of *SRY* in providing the primary signal for male development.

Further support of this conclusion involves an experiment using **transgenic mice.** These animals are produced from fertilized eggs injected with foreign DNA that is subsequently incorporated into the genetic composition of the developing embryo. In normal mice, a chromosome region designated *Sry* has been identified that is comparable to *SRY* in humans. When mouse DNA containing *Sry* is injected into normal XX mouse eggs, most of the offspring develop into males.

The question of how the product of this gene triggers development of embryonic gonadal tissue into testes rather than ovaries has been under investigation for 25 years. TDF functions as a *transcription factor,* a DNA-binding protein that interacts directly with regulatory sequences of other genes to stimulate their expression. Thus, while TDF behaves as a master switch that controls other genes downstream in the process of sexual differentiation, identifying TDF target genes has been difficult. One potential target for activation by TDF that has been extensively studied is the gene for **Müllerian inhibiting substance (MIS)**, [also called Mullerian inhibiting hormone, (MIH), or anti-Mullerian hormone]. Cells of the developing testes secrete MIS. As its name suggests, MIS protein causes regression (atrophy) of cells in the Müllerian duct. Degeneration of the duct prevents formation of the female reproductive tract.

Other autosomal genes are part of a cascade of genetic expression initiated by *SRY*. Examples include the human *SOX9* gene and the mouse homolog *Sox9*, which when activated by *SRY,* leads to the differentiation of cells that form the seminiferous tubules that contain male germ cells. In the mouse, fibroblast growth factor 9 *(Fgf9)* is upregulated in XY gonads. Testis development is completely blocked in gonads lacking *Fgf9,* and signs of ovarian development occur. Another gene, *SF1,* is involved in the regulation of enzymes affecting steroid metabolism. In mice, this gene is initially active in both the male and female bisexual genital ridge, persisting until the point in development when testis formation is apparent. At that time, its expression persists in males but is extinguished in females. Recent work using mice has

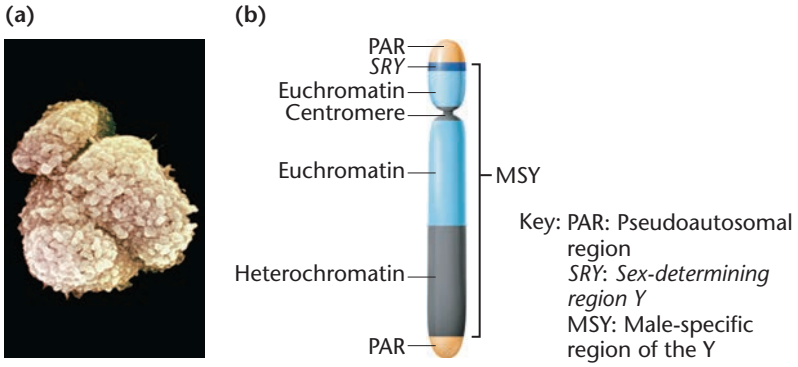


FIGURE 7.3 **(a)** Electron micrograph of the human Y chromosome (magnification × 35,000) and **(b)** regions of the Y chromosome.

* It is interesting to note that in chickens, a similar gene has recently been identified. Called *DMRTI*, it is located on the Z chromosome. This gene is the subject of Problem 29 in the Problems section at the end of the chapter.

suggested that testicular development may be actively repressed throughout the life of females by downregulating expression of specific genes. This is based on experiments showing that, in adult female mice, deletion of a gene *Foxl2,* which encodes a transcription factor, leads to transdifferentiation of the ovary into the testis.

In 2016, researchers at the University of Hawaii published novel work demonstrating that two genes in mice, *Sox9* and *Eif2s3y,* could substitute for the Y chromosome. *Sry* activates *Sox9,* and *Eif2s3y* has a homolog on the X chromosome (*Eif2s3x*). Transgenic mice with one X and no Y chromosome were generated. But in these mice, *Sry* was replaced with a transgenic copy of *Sox9* and made to overexpress *Eif2s3x* from an X chromosome, beyond the levels produced normally by the X and Y chromosomes. These males, lacking a Y chromosome, produced haploid male gametes. They did not produce mature sperm but yielded round spermatids that were used to fertilize an oocyte *in vitro,* resulting in viable offspring. This study demonstrated that *Sox9,* in the absence of *Sry,* and the *Eif2s3y* homolog, *Eif2s3x,* allow for male gamete development and initiation of spermatogenesis in the absence of a complete Y chromosome. While these two genes can result in male gametes that produce offspring through assisted reproductive technology, other genes are necessary to produce mature sperm, but nonetheless, experiments such as these are providing novel insights into the genetics of sex-determination pathways. Establishment of the link between these various genes and sex determination has brought us closer to a complete understanding of how males and females arise in humans, but much work remains to be done.

Findings by David Page and his many colleagues have provided a reasonably complete picture of the MSY region of the human Y chromosome. Page has spearheaded the detailed study of the Y chromosome for the past several decades. The MSY consists of about 23 million base pairs (23 Mb) and can be divided into three regions. The first region is the *X-transposed region.* It comprises about 15 percent of the MSY and was originally derived from the X chromosome in the course of human evolution (about 3 to 4 million years ago). The X-transposed region is 99 percent identical to region Xq21 of the modern human X chromosome. Two genes, both with X chromosome homologs, are present in this region.

Research by Page and others has also revealed that sequences called **palindromes**—sequences of base pairs that read the same but in the opposite direction on complementary strands—are present throughout the MSY. Recombination between palindromes on sister chromatids of the Y chromosome during replication is a mechanism used to repair mutations in the Y chromosome. This discovery has fascinating implications concerning how the Y chromosome may maintain its size and structure.

Another interesting finding is that the MSY of the human Y chromosome is very different in sequence structure than the MSY from chimpanzees. The study indicates that rapid evolution has occurred since separation of these species over 6 million years ago—a surprise given that primate sex chromosomes have been in existence for hundreds of millions of years. Over 30 percent of the chimpanzee MSY sequence has no homologous sequence in the human MSY. The chimpanzee MSY has lost many protein-coding genes compared to common ancestors but contains twice the number of palindromic sequences as the human MSY.

The second area of the MSY is designated the *X-degenerative region.* Comprising about 20 percent of the MSY, this region contains DNA sequences that are even more distantly related to those present on the X chromosome. The X-degenerative region contains 27 single-copy genes and a number of *pseudogenes* (genes whose sequences have degenerated sufficiently during evolution to render them nonfunctional). Twenty of the 27 genes located here share homology with counterparts on the X chromosome and evolved from genes on the X chromosome. One of these is the *SRY* gene, discussed earlier. Other X-degenerative genes that encode protein products are expressed ubiquitously in all tissues in the body, but *SRY* is expressed only in the testes.

The third area, the *ampliconic region,* contains about 30 percent of the MSY, including most of the genes closely associated with the development of testes. These genes lack counterparts on the X chromosome, and their expression is limited to the testes. There are 60 transcription units (genes that yield a product) divided among nine gene families in this region, most represented by multiple copies. Members of each family have nearly identical (>98 percent) DNA sequences. Each repeat unit is an **amplicon** and is contained within seven segments scattered across the euchromatic regions of both the short and long arms of the Y chromosome. Genes in the ampliconic region encode proteins specific to the development and function of the testes, and the products of many of these genes are directly related to fertility in males. It is currently believed that a great deal of male sterility in our population can be linked to mutations in these genes.

Until relatively recently it was thought that the Y chromosome only contributed to sex determination and male fertility. A recent area of investigation has involved the Y chromosome and paternal age. For many years, it has been known that maternal age is correlated with an elevated rate of offspring with chromosomal defects, including Down syndrome (see Chapter 8). Advanced

paternal age has now been associated with an increased risk in offspring of congenital disorders with a genetic basis, including certain cancers, schizophrenia, autism, and other conditions, collectively known as *paternal age effects (PAE)*. Studies in which the genomes of sperm have been sequenced have demonstrated the presence of specific PAE mutations including numerous ones on the Y chromosome. Evidence suggests that PAE mutations are positively selected for and result in an enrichment of mutant sperm over time.

Similarly, an analysis of blood samples and medical records for more than 6000 men in Sweden revealed a correlation between smoking and complete loss of the Y chromosome in blood cells. Y chromosome loss was also correlated to elevated cancer risk among male smokers, reduced expression of tumor-suppressor genes, and compromised immunity. This and other research provides further evidence that genes on the Y chromosome affect more than sex determination and male fertility.

This recent work has greatly expanded our picture of the genetic information carried by this unique chromosome. It clearly refutes the so-called *wasteland theory,* prevalent some 25 years ago, that depicted the human Y chromosome as almost devoid of genetic information other than a few genes that cause maleness. The knowledge we have gained provides the basis for a much clearer picture of how maleness is determined.

NOW SOLVE THIS

7.1 Campomelic dysplasia (CMD1) is a congenital human syndrome featuring malformation of bone and cartilage. It is caused by an autosomal dominant mutation of a gene located on chromosome 17. Consider the following observations in sequence, and in each case, draw whatever appropriate conclusions are warranted.

(a) Of those with the syndrome who are karyotypically 46,*XY*, approximately 75 percent are sex reversed, exhibiting a wide range of female characteristics.

(b) The nonmutant form of the gene, called *SOX9*, is expressed in the developing gonad of the XY male, but not the XX female.

(c) The *SOX9* gene shares 71 percent amino acid coding sequence homology with the Y-linked *SRY* gene.

(d) CMD1 patients who exhibit a 46,XX karyotype develop as females, with no gonadal abnormalities.

■ **HINT:** *This problem asks you to apply the information presented in this chapter to a real-life example. The key to its solution is knowing that some genes are activated and produce their normal product as a result of expression of products of other genes found on different chromosomes.*

7.3 The Ratio of Males to Females in Humans Is Not 1.0

The presence of heteromorphic sex chromosomes in one sex of a species but not the other provides a potential mechanism for producing equal proportions of male and female offspring. This potential depends on the segregation of the X and Y (or Z and W) chromosomes during meiosis, such that half of the gametes of the heterogametic sex receive one of the chromosomes and half receive the other one. As we learned in Section 7.2, small pseudoautosomal regions of pairing homology do exist at both ends of the human X and Y chromosomes, suggesting that the X and Y chromosomes do synapse and then segregate into different gametes. Provided that both types of gametes are equally successful in fertilization and that the two sexes are equally viable during development, a 1:1 ratio of male and female offspring should result.

The actual proportion of male to female offspring, referred to as the **sex ratio**, has been assessed in two ways. The **primary sex ratio (PSR)** reflects the proportion of males to females conceived in a population. The **secondary sex ratio** reflects the proportion of each sex that is born. The secondary sex ratio is much easier to determine but has the disadvantage of not accounting for any disproportionate embryonic or fetal mortality.

When the secondary sex ratio in the human population was determined in 1969 by using worldwide census data, it did not equal 1.0. For example, in the Caucasian population in the United States, the secondary ratio was a little less than 1.06, indicating that about 106 males were born for each 100 females. (In 1995, this ratio dropped to slightly less than 1.05.) In the African-American population in the United States, the ratio was 1.025. In other countries, the excess of male births is even greater than is reflected in these values. For example, in Korea, the secondary sex ratio was 1.15.

Despite these ratios, it is possible that the PSR is 1.0 and is altered between conception and birth. For the secondary ratio to exceed 1.0, then, prenatal female mortality would have to be greater than prenatal male mortality. However, when this hypothesis was first examined, it was deemed to be false. In a Carnegie Institute study, reported in 1948, the sex of approximately 6000 embryos and fetuses recovered from miscarriages and abortions was determined, and fetal mortality was actually higher in males. On the basis of the data derived from that study, the PSR in U.S. Caucasians was estimated to be 1.079, suggesting that more males than females are conceived in the human population.

To explain why, researchers considered the assumptions on which the theoretical ratio is based:

1. Because of segregation, males produce equal numbers of X- and Y-bearing sperm.
2. Each type of sperm has equivalent viability and motility in the female reproductive tract.
3. The egg surface is equally receptive to both X- and Y-bearing sperm.

No direct experimental evidence contradicts any of these assumptions.

A PSR favoring male conceptions remained dogma for many decades until, in 2015, a study using an extensive dataset was published determining that the PSR is indeeed 1.0, thus concluding that equal numbers of males and females are conceived. Among other parameters, the examination of the sex of 3-day-old and 6-day-old embryos conceived using assisted reproductive technology provided the most direct assessment. Following conception, however, mortality was then shown to fluctuate between the sexes, until at birth, more males than females are born. Thus, female mortality during embryonic and fetal development exceeds that of males. Clearly, this is a difficult topic to investigate but one of continued interest. For now, the most recent findings are convincing and contradict the earlier studies.

7.4 Dosage Compensation Prevents Excessive Expression of X-Linked Genes in Humans and Other Mammals

The presence of two X chromosomes in normal human females and only one X in normal human males is unique compared with the equal numbers of autosomes present in the cells of both sexes. On theoretical grounds alone, it is possible to speculate that this disparity should create a "genetic dosage" difference between males and females, with attendant problems, for all X-linked genes. There is the potential for females to produce twice as much of each product of all X-linked genes. The additional X chromosomes in both males and females exhibiting the various syndromes discussed earlier in this chapter are thought to compound this dosage problem. Embryonic development depends on proper timing and precisely regulated levels of gene expression. Otherwise, disease phenotypes or embryonic lethality can occur. In this section, we will describe research findings regarding X-linked gene expression that demonstrate a genetic mechanism of **dosage compensation** that balances the dose of X chromosome gene expression in females and males.

Barr Bodies

Murray L. Barr and Ewart G. Bertram's experiments with cats, as well as Keith Moore and Barr's subsequent study in humans, demonstrate a genetic mechanism in mammals that compensates for X chromosome dosage disparities. Barr and Bertram observed a darkly staining body in the interphase nerve cells of female cats that was absent in similar cells of males. In humans, this body can be easily demonstrated in female cells derived from the buccal mucosa (cheek cells) or in fibroblasts (undifferentiated connective tissue cells), but not in similar male cells (**Figure 7.4**). This highly condensed structure, about 1 μm in diameter, lies against the nuclear envelope of interphase cells, and it stains positively for a number of different DNA-binding dyes.

This chromosome structure, called a **sex chromatin body,** or simply a **Barr body,** is an inactivated X chromosome. Susumu Ohno was the first to suggest that the Barr body arises from one of the two X chromosomes. This hypothesis is attractive because it provides a possible mechanism for dosage compensation. If one of the two X chromosomes is inactive in the cells of females, the dosage of genetic information that can be expressed in males and females will be equivalent. Convincing, though indirect, evidence for this hypothesis comes from study of the sex-chromosome syndromes described earlier in this chapter. Regardless of how many X chromosomes a somatic cell possesses, all but one of them appear to be inactivated and can be seen as Barr bodies. For example, no Barr body is seen in the somatic cells of Turner 45,X females; one is seen in Klinefelter 47,XXY males; two in 47,XXX females; three in 48,XXXX females; and so on (**Figure 7.5**). Therefore, the number of Barr bodies follows an $N - 1$ rule, where N is the total number of X chromosomes present.

Although this apparent inactivation of all but one X chromosome increases our understanding of dosage compensation, it further complicates our perception of other

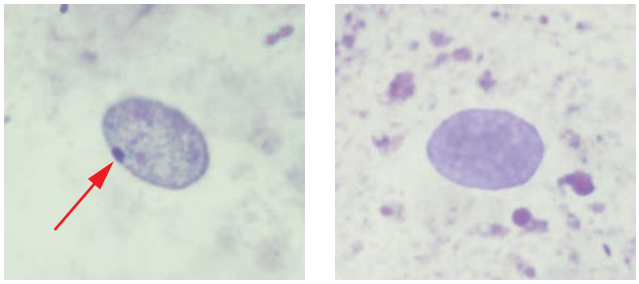


FIGURE 7.4 Photomicrographs comparing cheek epithelial cell nuclei from a male that fails to reveal Barr bodies (right) with a nucleus from a female that demonstrates a Barr body (indicated by the arrow in the left image). This structure, also called a sex chromatin body, represents an inactivated X chromosome.

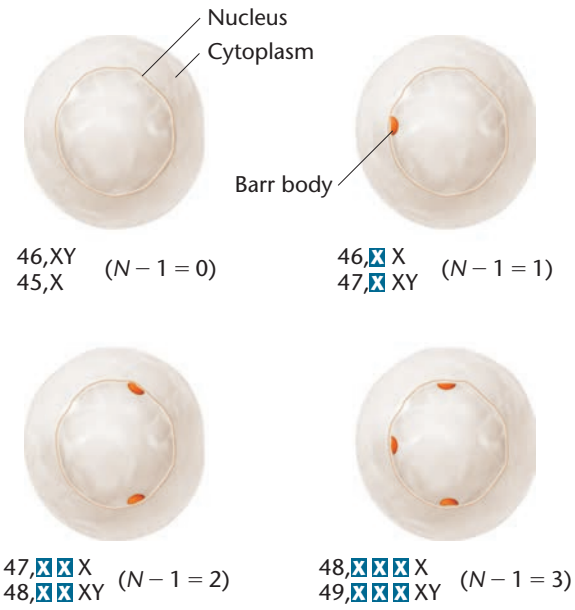


FIGURE 7.5 Occurrence of Barr bodies in various human karyotypes, where all X chromosomes except one ($N - 1$) are inactivated.

matters. For example, because one of the two X chromosomes is inactivated in normal human females, why then is the Turner 45,X individual not entirely normal? Why aren't females with the triplo-X and tetra-X karyotypes (47,XXX and 48,XXXX) completely unaffected by the additional X chromosome? Furthermore, in Klinefelter syndrome (47,XXY), X chromosome inactivation effectively renders the person 46,XY. Why aren't these males unaffected by the extra X chromosome in their nuclei?

One possible explanation is that chromosome inactivation does not normally occur in the very early stages of development of those cells destined to form gonadal tissues. Another possible explanation is that not all genes on each X chromosome forming a Barr body are inactivated. Recent studies have indeed demonstrated that as many as 15 percent of the human X chromosomal genes actually escape inactivation. Clearly, then, not every gene on the X requires inactivation. In either case, excessive expression of certain X-linked genes might still occur at critical times during development despite apparent inactivation of superfluous X chromosomes.

The Lyon Hypothesis

In mammalian females, one X chromosome is of maternal origin, and the other is of paternal origin. Which one is inactivated? Is the inactivation random? Is the same chromosome inactive in all somatic cells? In the early 1960s, Mary Lyon, Liane Russell, and Ernest Beutler independently proposed a hypothesis that answers these questions. They postulated that the inactivation of X chromosomes occurs randomly in somatic cells at a point early in embryonic development, most likely sometime during the blastocyst stage of development. Once inactivation has occurred, all descendant cells have the same X chromosome inactivated as their initial progenitor cell.

This explanation, which has come to be called the **Lyon hypothesis,** was initially based on observations of female mice heterozygous for X-linked coat-color genes. The pigmentation of these heterozygous females was mottled, with large patches expressing the color allele on one X and other patches expressing the allele on the other X. This is the phenotypic pattern that would be expected if different X chromosomes were inactive in adjacent patches of cells. Similar mosaic patterns occur in the black and yellow-orange patches of female tortoiseshell and calico cats (**Figure 7.6**). Such X-linked coat-color patterns do not

(a)

(b)

FIGURE 7.6 (a) The random distribution of orange and black patches in a calico cat illustrates the Lyon hypothesis. The white patches are due to another gene, distinguishing calico cats from tortoiseshell cats (b), which lack the white patches.

occur in male cats because all their cells contain the single maternal X chromosome and are therefore hemizygous for only one X-linked coat-color allele.

The most direct evidence in support of the Lyon hypothesis comes from studies of gene expression in clones of human fibroblast cells. Individual cells are isolated following biopsy and cultured *in vitro*. A culture of cells derived from a single cell is called a **clone.** The synthesis of the enzyme glucose-6-phosphate dehydrogenase (G6PD) is controlled by an X-linked gene. Numerous mutant alleles of this gene have been detected, and their gene products can be differentiated from the wild-type enzyme by their migration pattern in an electrophoretic field.

Fibroblasts have been taken from females heterozygous for different allelic forms of *G6PD* and studied. The Lyon hypothesis predicts that if inactivation of an X chromosome occurs randomly early in development, and thereafter all progeny cells have the same X chromosome inactivated as their progenitor, such a female should show two types of clones, each containing only one electrophoretic form of *G6PD,* in approximately equal proportions. This prediction has been confirmed experimentally, and studies involving modern techniques in molecular biology have clearly established that X chromosome inactivation occurs.

One ramification of X-inactivation is that mammalian females are mosaics for all heterozygous X-linked alleles—some areas of the body express only the maternally derived alleles, and others express only the paternally derived alleles. An especially interesting example involves **red-green color blindness**, an X-linked recessive disorder. In humans, hemizygous males are fully color-blind in all retinal cells. However, heterozygous females display mosaic retinas, with patches of defective color perception and surrounding areas with normal color perception. In this example, random inactivation of one or the other X chromosome early in the development of heterozygous females has led to these phenotypes.

The Mechanism of Inactivation

The least understood aspect of the Lyon hypothesis is the mechanism of X chromosome inactivation. Somehow, either DNA, the attached histone proteins, or both DNA and histone proteins, are chemically modified, silencing most genes that are part of that chromosome. Once silenced, a memory is created that keeps the same homolog inactivated following chromosome replications and cell divisions. Such a process, whereby expression of genes on one homolog, but not the other, is affected, is referred to as **imprinting**. This term also applies to a number of other examples in which genetic information is modified and gene expression is repressed. Collectively, such events are part of the growing field of **epigenetics** (see Chapter 19).

NOW SOLVE THIS

7.2 Carbon Copy (CC), the first cat produced from a clone, was created from an ovarian cell taken from her genetic donor, Rainbow, a calico cat. The diploid nucleus from the cell was extracted and then injected into an enucleated egg. The resulting zygote was then allowed to develop in a petri dish, and the cloned embryo was implanted in the uterus of a surrogate mother cat, who gave birth to CC. CC's surrogate mother was a tabby (see the photo below). Geneticists were very interested in the outcome of cloning a calico cat because they were not certain if the cloned cat would have patches of orange and black, just orange, or just black. Taking into account the Lyon hypothesis, explain the basis of the uncertainty. Would you expect CC to appear identical to Rainbow? Explain why or why not.

Carbon Copy with her surrogate mother.

■ **HINT:** *This problem involves an understanding of the Lyon hypothesis. The key to its solution is to realize that the donor nucleus was from a differentiated ovarian cell of an adult female cat, which itself had inactivated one of its X chromosomes.*

Ongoing investigations are beginning to clarify the mechanism of inactivation. A region of the mammalian X chromosome is the major control unit. This region, located on the proximal end of the p arm in humans, is called the **X-inactivation center (*Xic*)**, and its genetic expression *occurs only on the X chromosome that is inactivated*. The *Xic* is about 1 Mb (10^6 base pairs) in length and is known to contain several putative regulatory units and four genes. One of these, *X-inactive specific transcript (XIST)*, which encodes a long noncoding RNA (lncRNA), is now known to be a critical gene for X-inactivation.

Interesting observations have been made regarding the XIST lncRNA, many coming from experiments that focused on the equivalent gene in the mouse (*Xist*). First, the RNA product is quite large and does not encode a protein, and thus is not translated. The *Xist* transcript is an example of a lncRNA. An *Xist* lncRNA recruits a protein complex that

silences transcription at the epigenetic level. The role of lncRNAs in gene expression regulation will be discussed in greater detail in Chapter 19. The *Xist* lncRNAs spread over and coat the X chromosome *bearing the gene that produced them*. Two other noncoding genes at the *Xic* locus, *Tsix* (an antisense partner of *Xist*) and *Xite,* are also believed to play important roles in X-inactivation. It is thought that both *Xist* lncRNA expression and X-linked gene silencing are controlled by a small number of genes that are not inactivated in females.

Another observation is that transcription of *Xist* initially occurs at low levels on all X chromosomes. As the inactivation process begins, however, transcription continues, and is enhanced, only on the X chromosome that becomes inactivated. In addition to silencing genes on the inactivated X chromosome, there are 3D changes in the structure of the inactivated X chromosome that exclude RNA polymerase II from binding to transcription complexes. Recent work has revealed that the *Xist* lncRNA plays a role in these 3D changes in chromosome structure, which in turn help *Xist* to spread and inactivate genes across the X chromosome.

Interesting questions remain regarding imprinting and inactivation. For example, in cells with more than two X chromosomes, what sort of "counting" mechanism exists that designates all but one X chromosome to be inactivated? Studies by Jeannie T. Lee and colleagues suggest that maternal and paternal X chromosomes must first pair briefly and align at their *Xic* loci as a mechanism for counting the number of X chromosomes prior to X-inactivation [**Figure 7.7(a)**]. Using mouse embryonic stem cells, Lee's group deleted the *Tsix* gene contained in the *Xic* locus. This deletion blocked X–X pairing and resulted in chaotic inactivation of 0, 1, or 2 X chromosomes [**Figure 7.7(b)**]. Lee and colleagues provided further evidence for the role of the *Xic* locus in chromosome counting by adding copies of genetically engineered non-X chromosomes containing multiple copies of *Tsix* or *Xite.* (These are referred to as **transgenes** because they are artificially introduced into the organism.) This experimental procedure effectively blocked X–X pairing and prevented X chromosome inactivation [**Figure 7.7(c)**].

Other genes and protein products are being examined for their role in X chromosome pairing and counting. Recent studies by Lee and colleagues have provided evidence that the inactivated X chromosome must associate with regions at the periphery of the nucleus to maintain a state of silenced gene expression. Indeed, in a majority of human female somatic cells the inactivated X chromosome, present as a Barr body, is observed attached to the nuclear envelope.

Many questions remain. What "blocks" the *Xic* locus of the active chromosome, preventing further transcription of *Xist*? How does imprinting impart a memory such that inactivation of the same X chromosome or chromosomes is subsequently maintained in progeny cells, as the Lyon hypothesis calls for? Whatever the answers to these questions, scientists have taken exciting steps toward understanding how dosage compensation is accomplished in mammals.

Finally, modern applications of genomic analysis (which you will learn more about in Chapter 22) have enabled researchers to compare gene expression changes between males and females on a genome-wide scale. Such

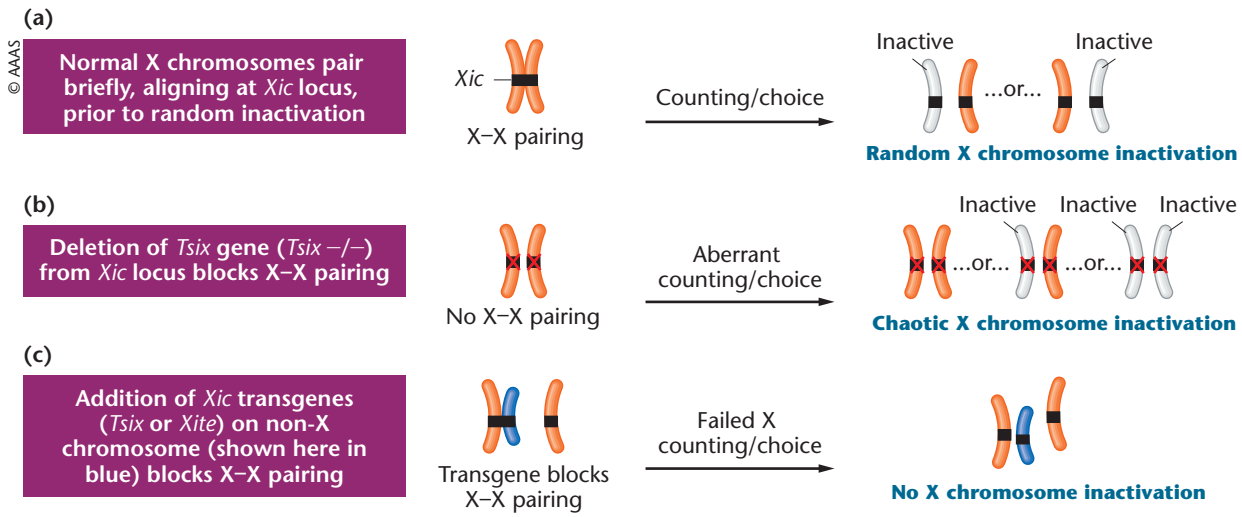


FIGURE 7.7 (a) Transient pairing of X chromosomes may be required for initiating X-inactivation. (b) Deleting the *Tsix* gene of the *Xic* locus prevents X-X pairing and leads to chaotic X-inactivation. (c) Blocking X-X pairing by addition of *Xic*-containing transgenes blocks X-X pairing and prevents X-inactivation.

work is revealing examples of sex-biased gene expression—genes that are expressed predominantly in one sex or another (or at a higher level in one sex). Undoubtedly these studies will provide additional insight about silenced genes and activated genes that contribute to sex determination.

7.5 The Ratio of X Chromosomes to Sets of Autosomes Can Determine Sex

We now discuss two interesting cases where the Y chromosome does not play a role in sex determination. First, in the fruit fly, *Drosophila melanogaster*, even though most males contain a Y chromosome, the Y plays no role. Second, in the roundworm, *Caenorhabditis elegans*, the organism lacks a Y chromosome altogether. In both cases, we shall see that the critical factor is the ratio of X chromosomes to the number of sets of autosomes.

D. melanogaster

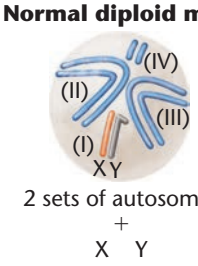
Because males and females in *Drosophila melanogaster* (and other *Drosophila* species) have the same general sex-chromosome composition as humans (males are XY and females are XX), we might assume that the Y chromosome also causes maleness in these flies. However, the elegant work of Calvin Bridges in 1921 showed this not to be true. His studies of flies with quite varied chromosome compositions led him to the conclusion that the Y chromosome is not involved in sex determination in this organism. Instead, Bridges proposed that the X chromosomes and autosomes together play a critical role in sex determination.

Bridges' work can be divided into two phases: (1) A study of offspring resulting from nondisjunction of the X chromosomes during meiosis in females and (2) subsequent work with progeny of females containing three copies of each chromosome, called triploid ($3n$) females. As we have seen previously in this chapter (and as you will see in Figure 8.1), nondisjunction is the failure of paired chromosomes to segregate or separate during the anaphase stage of the first or second meiotic divisions. The result is the production of two types of abnormal gametes, one of which contains an extra chromosome ($n + 1$) and the other of which lacks a chromosome ($n - 1$). Fertilization of such gametes with a haploid gamete produces ($2n + 1$) or ($2n - 1$) zygotes. As in humans, if nondisjunction involves the X chromosome, in addition to the normal complement of autosomes, both an XXY and an X0 sex-chromosome composition may result. (The "0" signifies that neither a second X nor a Y chromosome is present, as occurs in X0 genotypes of individuals with Turner syndrome.)

Contrary to what was later discovered in humans, Bridges found that the XXY flies were normal females and the X0 flies were sterile males. The presence of the Y chromosome in the XXY flies did not cause maleness, and its absence in the X0 flies did not produce femaleness. From these data, Bridges concluded that the Y chromosome in *Drosophila* lacks male-determining factors, but since the X0 males were sterile, it does contain genetic information essential to male fertility.

Bridges was able to clarify the mode of sex determination in *Drosophila* by studying the progeny of triploid females ($3n$), which have three copies each of the haploid complement of chromosomes. *Drosophila* has a haploid number of 4, thereby possessing three pairs of autosomes in addition to its pair of sex chromosomes. Triploid females apparently originate from rare diploid eggs fertilized by normal haploid sperm. Triploid females have heavy-set bodies, coarse bristles, and coarse eyes, and they may be fertile. Because of the odd number of each chromosome (3), during meiosis, a variety of different chromosome complements are distributed into gametes that give rise to offspring with a variety of abnormal chromosome constitutions. Correlations between the sexual morphology and chromosome composition, along with Bridges' interpretation, are shown in **Figure 7.8**.

Bridges realized that the critical factor in determining sex is the ratio of X chromosomes to the number of haploid



Chromosome formulation	Ratio of X chromosomes to autosome sets	Sexual morphology
3X:2A	1.5	Metafemale
3X:3A	1.0	Female
2X:2A	1.0	Female
3X:4A	0.75	Intersex
2X:3A	0.67	Intersex
X:2A	0.50	Male
XY:2A	0.50	Male
XY:3A	0.33	Metamale

FIGURE 7.8 The ratios of X chromosomes to sets of autosomes and the resultant sexual morphology seen in *Drosophila melanogaster.*

sets of autosomes (A) present. Normal (2X:2A) and triploid (3X:3A) females each have a ratio equal to 1.0, and both are fertile. As the ratio exceeds unity (3X:2A, or 1.5, for example), what was once called a *superfemale* is produced. Because such females are most often inviable, they are now more appropriately called **metafemales.**

Normal (XY:2A) and sterile (X0:2A) males each have a ratio of 1:2, or 0.5. When the ratio decreases to 1:3, or 0.33, as in the case of an XY:3A male, infertile **metamales** result. Other flies recovered by Bridges in these studies had an (X:A) ratio intermediate between 0.5 and 1.0. These flies were generally larger, and they exhibited a variety of morphological abnormalities and rudimentary bisexual gonads and genitalia. They were invariably sterile and expressed both male and female morphology, thus being designated as **intersexes.**

Bridges' results indicate that in *Drosophila*, factors that cause a fly to develop into a male are not located on the sex chromosomes but are instead found on the autosomes. Some female-determining factors, however, are located on the X chromosomes. Thus, with respect to primary sex determination, male gametes containing one of each autosome plus a Y chromosome result in male offspring not because of the presence of the Y but because they fail to contribute an X chromosome. This mode of sex determination is explained by the **genic balance theory.** Bridges proposed that a threshold for maleness is reached when the X:A ratio is 1:2 (X:2A), but that the presence of an additional X (XX:2A) alters the balance and results in female differentiation.

Numerous genes involved in sex determination in *Drosophila* have been identified. The recessive autosomal gene *transformer* (*tra*), discovered over 50 years ago by Alfred H. Sturtevant, clearly demonstrated that a single autosomal gene could have a profound impact on sex determination. Females homozygous for *tra* are transformed into sterile males, but homozygous males are unaffected. More recently, another gene, *Sex-lethal* (*Sxl*), has been shown to play a critical role, serving as a "master switch" in sex determination. Activation of the X-linked *Sxl* gene, which relies on a ratio of X chromosomes to sets of autosomes that equals 1.0, is essential to female development. In the absence of activation—as when, for example, the X:A ratio is 0.5—male development occurs.

Although it is not yet exactly clear how this ratio influences the *Sxl* locus, we do have some insights into the question. The *Sxl* locus is part of a hierarchy of gene expression and exerts control over other genes, including *tra* (discussed in the previous paragraph) and *dsx (doublesex)*. The wild-type allele of *tra* is activated by the product of *Sxl* only in females and in turn influences the expression of *dsx*. Depending on how the initial RNA transcript of *dsx* is processed the resultant dsx protein activates either male- or female-specific genes required for sexual differentiation. It is interesting to note that *dsx* homologs have been found in all mammals, thus emphasizing a role for this gene in gonad development.

Each step in this regulatory cascade requires a form of processing called **RNA splicing,** in which portions of the RNA are removed and the remaining fragments are "spliced" back together prior to translation into a protein. In the case of the *Sxl* gene, the RNA transcript may be spliced in different ways, a phenomenon called **alternative splicing.** A different RNA transcript is produced in females than in males. In potential females, the transcript is active and initiates a cascade of regulatory gene expression, ultimately leading to female differentiation. In potential males, the transcript is inactive, leading to a different pattern of gene activity, whereby male differentiation occurs. We will return to this topic in Chapter 18, where alternative splicing is again addressed as one of the mechanisms involved in the regulation of genetic expression in eukaryotes.

Caenorhabditis elegans

The nematode worm *C. elegans* [**Figure 7.9(a)**] has become a popular organism in genetic studies, particularly for investigating the genetic control of development. Its usefulness is

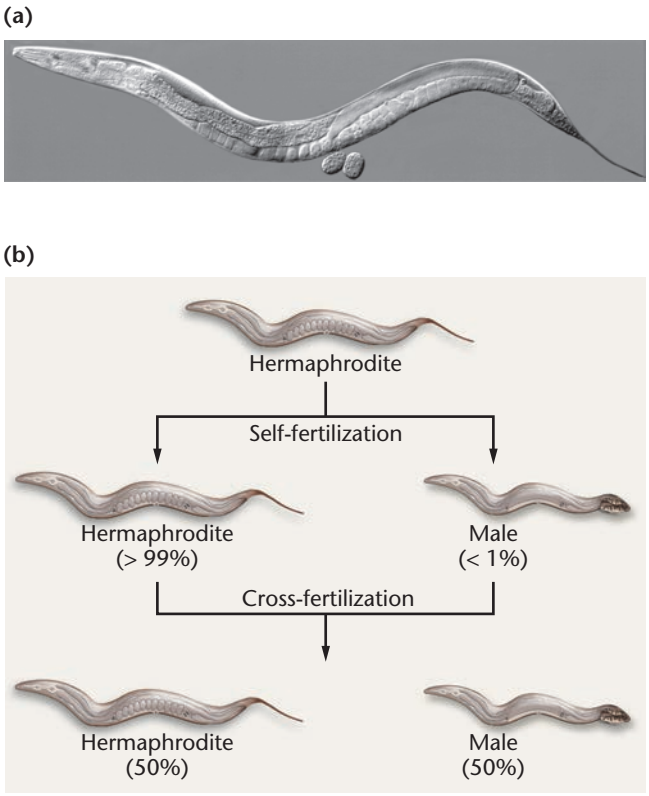


FIGURE 7.9 (a) Photomicrograph of a hermaphroditic nematode, *C. elegans*; (b) the outcomes of self-fertilization in a hermaphrodite, and a mating of a hermaphrodite and a male worm.

MODERN APPROACHES TO UNDERSTANDING GENE FUNCTION

Drosophila Sxl Gene Induces Female Development

Scientists have investigated a critical step involved in determining how germ-cell development, the ability to form either sperm or egg cells, is regulated in fruit flies. As discussed in this chapter, in *Drosophila* the *Sxl* gene encodes a protein that binds to RNA and regulates RNA splicing events. While the molecular mechanism that initiates female germ-cell fate is not known, researchers have hypothesized that the *Sxl* gene product might provide the critical switch that regulates this process. To test this hypothesis, they expressed *Sxl* in **primordial germ cells (PGCs)**, undifferentiated diploid germ cells from XY male flies that would normally become sperm. *Sxl*-containing PGCs were then implanted into the ovaries of female flies and induced to enter oogenesis.

Results:

The table above summarizes *Sxl* implantation results. Implanted cells were tracked to determine whether or not they produced oocytes and whether such oocytes were capable of being fertilized and producing progeny. As shown in the table, XY donor PGCs lacking *Sxl* (XY-nullo *Sxl*) did not produce oocytes when transplanted into ovaries. When XY PGCs containing the *Sxl* gene were transplanted, 72 percent produced eggs, and these eggs were capable of being fertilized and producing progeny! Thus, the addition of a single gene to Y chromosome–containing cells destined to become sperm is sufficient to change the fate of these cells to induce female germ-cell development. Various control experiments were conducted, including XY donor cells with a mutant, nonfunction version of *Sxl* (XY-nullo *Sxl*).

Donor PGCs	Number of Female Adults with Transplanted Germ-line Cells	Number of Female Adults with Transplanted Germ-line Cells Executing Oogenesis	Number of Female Adults with Transplanted Germ-line Cells Producing Progeny
XY	13	0 (0%)	0
XY + *Sxl*	18	13 (72%)	8
XY-nullo *Sxl*	15	0 (0%)	0
XX	25	25 (100%)	25

Conclusion:

Sxl functions to initiate development of female germ cells in *Drosophila*. Many questions remain, such as how *Sxl* is activated in female germ cells and which RNA molecules are affected by the *Sxl* gene product in female germ cells. Nonetheless, this result is very exciting because germ-line sexual development is thought to be highly conserved between animal species. Understanding how genes such as *Sxl* regulate germ-cell development in *Drosophila* is expected to help scientists understand key aspects of gamete development in mammals, including humans.

References:

Van Doren, M. (2011). Determining sexual identity. *Science* 333:829–830.
Hashiyama, K., et al. (2011). *Drosophila* sex lethal gene initiates female development in germline progenitors. *Science* 333:885–888

Questions to Consider:

1. Which of the four experiments shown in the above table are controls?
2. Discuss the role of each control experiment. Are the results what you would predict?

based on the fact that adults consist of approximately 1000 cells, the precise lineage of which can be traced back to specific embryonic origins. There are two sexual phenotypes in these worms: males, which have only testes, and hermaphrodites, which contain both testes and ovaries. During larval development of hermaphrodites, testes form that produce sperm, which is stored. Ovaries are also produced, but oogenesis does not occur until the adult stage is reached several days later. The eggs that are produced are fertilized by the stored sperm in a process of self-fertilization.

The outcome of this process is quite interesting [**Figure 7.9(b)**]. The vast majority of organisms that result are hermaphrodites, like the parental worm; less than 1 percent of the offspring are males. As adults, males can mate with hermaphrodites, producing about half male and half hermaphrodite offspring.

The genetic signal that determines maleness in contrast to hermaphroditic development is provided by genes located on both the X chromosome and autosomes. *C. elegans* lacks a Y chromosome altogether—hermaphrodites have two X chromosomes, while males have only one X chromosome. It is believed that, as in *Drosophila*, it is the ratio of X chromosomes to the number of sets of autosomes that ultimately determines the sex of these worms. A ratio of 1.0 (two X chromosomes and two copies of each autosome) results in hermaphrodites, and a ratio of 0.5 results in males. The absence of a heteromorphic Y chromosome is not uncommon in organisms.

7.6 Temperature Variation Controls Sex Determination in Many Reptiles

We conclude this chapter by discussing several cases involving reptiles, in which the environment—specifically temperature—has a profound influence on sex determination. In contrast to **chromosomal, or genotypic, sex determination (CSD or GSD),** in which sex is determined genetically (as is true of all examples thus far presented in the chapter), the cases that we will now discuss are categorized as **temperature-dependent sex determination (TSD)**. In recent years this topic has received more attention because rapid climate change may influence the sex ratio of certain species and thus threaten their existence in the future.

In many species of reptiles, sex is predetermined at conception by sex-chromosome composition, as is the case in many organisms already considered in this chapter. For example, in many snakes, including vipers, a ZZ/ZW mode is in effect, in which the female is the heterogametic sex (ZW). However, in boas and pythons, it is impossible to distinguish one sex chromosome from the other in either sex. In many lizards, both the XX/XY and ZZ/ZW systems are found, depending on the species.

In still other reptilian species, however, TSD is the norm, including all crocodiles, most turtles, and some lizards, where sex determination is achieved according to the incubation temperature of eggs during a critical period of embryonic development. Three distinct patterns of TSD emerge (cases I–III in **Figure 7.10**). In case I, low temperatures yield 100 percent females, and high temperatures yield 100 percent males. Just the opposite occurs in case II. In case III, low *and* high temperatures yield 100 percent females, while intermediate temperatures yield various proportions of males. The third pattern is seen in various species of crocodiles, turtles, and lizards, although other members of these groups are known to exhibit the other patterns.

Two observations are noteworthy. First, in all three patterns, certain temperatures result in both male and female offspring; second, this pivotal temperature T_P range is fairly narrow, usually spanning less than 5°C, and sometimes only 1°C. The central question raised by these observations is: What are the metabolic or physiological parameters affected by temperature that lead to the differentiation of one sex or the other?

The answer is thought to involve steroids (mainly estrogens) and the enzymes involved in their synthesis. Studies clearly demonstrate that the effects of temperature on estrogens, androgens, and inhibitors of the enzymes controlling their synthesis are involved in the sexual differentiation of ovaries and testes. One enzyme in particular, **aromatase**, converts androgens (male hormones such as testosterone) to estrogens (female hormones such as estradiol). The activity of this enzyme is correlated with the pathway of reactions that occurs during gonadal differentiation activity and is high in developing ovaries and low in developing testes.

In 2016, the first gene linked to TSD, *CIRBP* (cold-inducible RNA-binding protein), was identified in common snapping turtles. Increased expression of this gene occurs within 24 hours when turtle eggs are shifted from a male-determining temperature (MT) to a female-determining temperature (FT). Shortly after increased expression of *CIRBP*, several genes involved in development of the ovary are activated while genes involved in testis development are repressed. Researchers studying *CIRBP*

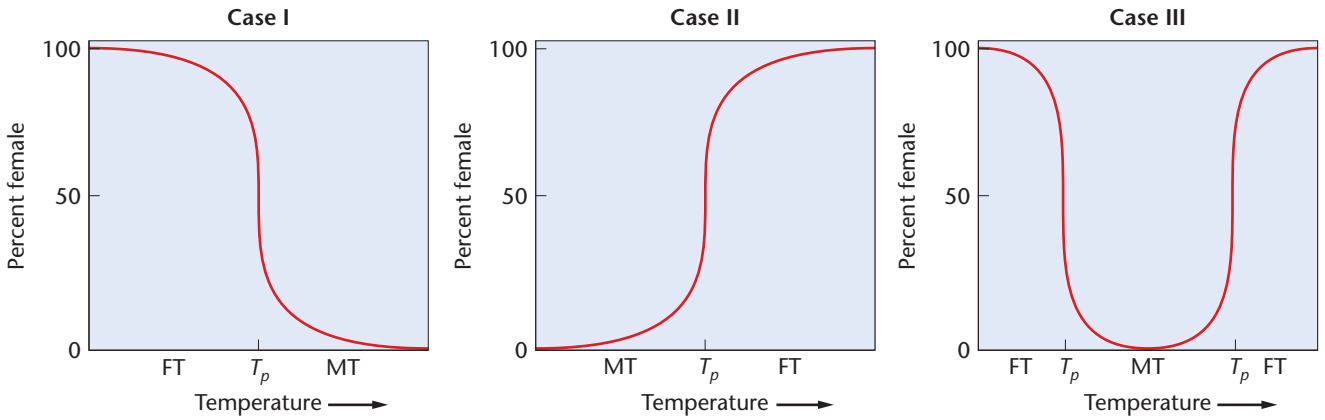
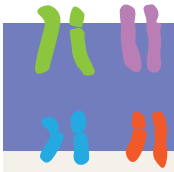


FIGURE 7.10 Three different patterns of temperature-dependent sex determination (TSD) in reptiles, as described in the text. The relative pivotal temperature T_p is crucial to sex determination during a critical point during embryonic development. FT = Female-determining temperature; MT = Male-determining temperature.

also identified an SNP in the gene, which is more common in male than female turtles. Recently, however, researchers discovered that the sex of Australian lizards (*Pogona vitticeps*) can be determined by both sex chromosomes and by the temperature at which *P. vitticeps* eggs are incubated. *P. vitticeps* has a female heterogametic system (ZW) and a male homogametic (ZZ) system. But this research revealed that in the wild nearly 20 percent of ZZ individuals are female and not male. Subsequently it was determined that ZZ females in the wild develop from eggs incubated at elevated temperatures— effectively causing a sex reversal. Hence, this is a species in which sex chromosomes and temperature impact sex determination. Further, experiments show that normal males mated to sex-reversed females produce offspring whose sex is determined solely by temperature. This raises interesting questions about the potential impacts of climate change on sex reversal and sex ratios in this species.



GENETICS, ETHICS, AND SOCIETY

A Question of Gender: Sex Selection in Humans

Throughout history, people have attempted to influence the gender of their unborn offspring by following varied and sometimes bizarre procedures. In medieval Europe, prospective parents would place a hammer under the bed to help them conceive a boy, or a pair of scissors, to conceive a girl. Other practices were based on the ancient belief that semen from the right testicle created male offspring and that from the left testicle created females. In some cultures, efforts to control the sex of offspring has had a darker side—female infanticide. In ancient Greece, the murder of female infants was so common that the male:female ratio in some areas approached 4:1. In some parts of rural India, female infanticide continued up to the 1990s. In 1997, the World Health Organization reported that about 50 million women were "missing" in China, likely because of selective abortion of female fetuses and institutionalized neglect of female children. In recent times, amniocentesis and ultrasound testing, followed by sex-specific abortion, have replaced much of the traditional female infanticide.

New genetic and reproductive technologies offer parents ways to select their children's gender prior to implantation of the embryo in the uterus—methods called *preimplantation gender selection (PGS)*. Following *in vitro* fertilization, embryos can be biopsied and assessed for gender. Only sex-selected embryos are then implanted.

The new PGS methods raise a number of legal and ethical issues. Some people feel that prospective parents have the right to use sex-selection techniques as part of their fundamental procreative liberty. Proponents state that PGS will reduce the suffering of many families. For example, people at risk for transmitting X-linked diseases such as hemophilia or Duchenne muscular dystrophy would be able to enhance their chance of conceiving a female child, who would not express the disease.

The majority of people who undertake PGS, however, do so for nonmedical reasons—to "balance" their families. One argument in favor of this use is that the intentional selection of the sex of an offspring may reduce overpopulation and economic burdens for families who would repeatedly reproduce to get the desired gender. Also, PGS may increase the happiness of both parents and children, as the children would be more "wanted."

On the other hand, some argue that PGS serves neither the individual nor the common good. They argue that PGS is inherently sexist, having its basis in the idea that one sex is superior to the other, and leads to linking a child's worth to gender. Other critics fear that social approval of PGS will open the door to other genetic manipulations of children's characteristics. It is difficult to predict the full effects that PGS will bring to the world. But the gender-selection genie is now out of the bottle and is unwilling to return.

Your Turn

Take time, individually or in groups, to answer the following questions. Investigate the references and links to help you understand some of the ethical issues that surround the topic of gender selection.

1. A generally accepted moral and legal concept is that of reproductive autonomy—the freedom to make individual reproductive decisions without external interference. Are there circumstances in which reproductive autonomy should be restricted?

   This question is explored in a series of articles in the American Journal of Bioethics, Vol. 1 (2001). *See the article by* J. A. Robertson on pages 2–9 *for a summary of the moral and legal issues surrounding PGS. Also see* Kalfoglou, A. L., et al. (2013). Ethical arguments for and against sperm sorting for non-medical sex selection: a review. *Repro. BioMed. Online* 26:231–239 (http://www.rbmojournal.com/article/S1472-6483(12)00692-X/fulltext#).

2. If safe and efficient methods of PGS were available, would you use them to help you with family planning? Under what circumstances might you use them?

   A discussion of PGS ethics and methods is presented in Use of reproductive technology for sex selection for nonmedical reasons, Ethics Committee of the American Society for Reproductive Medicine (2015) (http://www.reproductivefacts.org/globalassets/asrm/asrm-content/news-and-publications/ethics-committee-opinions/use_of_reproductive_technology_for_sex_selection_for_nonmedical_reasons-pdfmembers.pdf.).

CASE STUDY Is it a boy or a girl?

Gender is someone's conscious and unconscious feelings of belonging to one sex or another. Each year, about 1 in 4500 children are born with a disorder involving sexual development, where the chromosomal, gonadal, or anatomical sex is atypical. Here we will consider two similar cases with different outcomes. In case 1, a 2-year-old child displayed a mosaic chromosome composition of 45,X/46,XY, with one ovary, one testis, a uterus, and ambiguous genitalia. In case 2, a fetus was diagnosed with a mosaic chromosome composition of 46,XX/47,XXY, and after birth, also displayed one testis, one ovary, a uterus, and ambiguous genitalia. The child in case 1 was adopted from an orphanage and raised as a girl. After consultation with the medical team, the parents decided to continue raising the child as a girl and requested surgery that would completely feminize the child. In case 2, the parents decided to forego treatment and let the child make the choice about gender later in life and to remain neutral about the child's present condition. These cases raise questions about sex determination and the ethics of sex and gender assignment.

1. In humans, what is the role of the MSY region of the Y chromosome in sex determination and gender development?
2. Compare and contrast the ethical decisions faced by the parents in both cases 1 and 2. Should parents be allowed to make the decision about the gender of their child? If not, at what age should the child be allowed to make this decision?

See Kipnis, K., and Diamond, M. (1998). Pediatric ethics and the surgical assignment of sex. *J. Clin. Ethics* 9(4):398–410.

Summary Points

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1. Sexual reproduction depends on the differentiation of male and female structures responsible for the production of male and female gametes, which in turn is controlled by specific genes, most often housed on specific sex chromosomes.
2. Specific sex chromosomes contain genetic information that controls sex determination and sexual differentiation.
3. The presence of a Y chromosome that contains an intact *SRY* gene is responsible for causing maleness in humans and other mammals.
4. In humans, the most current study of the primary sex ratio shows that equal numbers of males and females are conceived, but that more males than females are born.
5. In mammals, female somatic cells randomly inactivate one of two X chromosomes during early embryonic development, a process important for balancing the expression of X chromosome-linked genes in males and females.
6. The Lyon hypothesis states that early in development, inactivation of either the maternal or paternal X chromosome occurs in each cell, and that all progeny cells subsequently inactivate the same chromosome. Mammalian females thus develop as mosaics for the expression of heterozygous X-linked alleles.
7. Although chromosome composition determines the sex of some reptiles, many others show that temperature-dependent effects during egg incubation are critical for sex determination.

INSIGHTS AND SOLUTIONS

1. In *Drosophila,* the X chromosomes may become attached to one another ($\widehat{XX}$) such that they always segregate together. Some flies thus contain a set of attached X chromosomes plus a Y chromosome.

   (a) What sex would such a fly be? Explain why this is so.

   (b) Given the answer to part (a), predict the sex of the offspring that would occur in a cross between this fly and a normal one of the opposite sex.

   (c) If the offspring described in part (b) are allowed to interbreed, what will be the outcome?

   Solution:

   (a) The fly will be a female. The ratio of X chromosomes to sets of autosomes—which determines sex in *Drosophila*—will be 1.0, leading to normal female development. The Y chromosome has no influence on sex determination in *Drosophila*.

   (b) All progeny flies will have two sets of autosomes along with one of the following sex-chromosome compositions:

   (1) $\widehat{XX}X \rightarrow$ a metafemale with 3 X's (called a trisomic)

   (2) $\widehat{XX}Y \rightarrow$ a female like her mother

   (3) XY → a normal male

   (4) YY → no development occurs

   (c) A stock will be created that maintains attached-X females generation after generation.

2. The Xg cell-surface antigen is coded for by a gene located on the X chromosome. No equivalent gene exists on the Y chromosome. Two codominant alleles of this gene have been identified: *Xg1* and *Xg2*. A woman of genotype *Xg2/Xg2* bears children with a man of genotype *Xg1/Y*, and they produce a son with Klinefelter syndrome of genotype *Xg1/Xg2/Y*. Using proper genetic terminology, briefly explain how this individual was generated. In which parent and in which meiotic division did the mistake occur?

   Solution: Because the son with Klinefelter syndrome is *Xg1/Xg2/Y*, he must have received both the *Xg1* allele and the Y chromosome from his father. Therefore, nondisjunction must have occurred during meiosis I in the father.

Problems and Discussion Questions

Mastering Genetics Visit for instructor-assigned tutorials and problems.

1. **HOW DO WE KNOW?** In this chapter, we have focused on sex differentiation, sex chromosomes, and genetic mechanisms involved in sex determination. At the same time, we found many opportunities to consider the methods and reasoning by which much of this information was acquired. From the explanations given in the chapter, you should answer the following fundamental questions?

   (a) How do we know whether or not a heteromorphic chromosome such as the Y chromosome plays a crucial role in the determination of sex?
   (b) How do we know that in humans the X chromosomes play no role in human sex determination, while the Y chromosome causes maleness and its absence causes femaleness?
   (c) How do we know that *Drosophila* utilizes a different sex-determination mechanism than mammals, even though it has the same sex-chromosome compositions in males and females?
   (d) How do we know that X chromosomal inactivation of either the paternal or maternal homolog is a random event during early development in mammalian females?

2. **CONCEPT QUESTION** Review the Chapter Concepts list on p. 151. These all center around sex determination or the expression of genes encoded on sex chromosomes. Write a short essay that discusses sex chromosomes as they contrast with autosomes.

3. Distinguish between the concepts of sexual differentiation and sex determination.

4. Contrast the XX/XY and XX/X0 modes of sex determination.

5. Describe the major difference between sex determination in *Drosophila* and in humans.

6. How do mammals, including humans, solve the "dosage problem" caused by the presence of an X and Y chromosome in one sex and two X chromosomes in the other sex?

7. The phenotype of an early-stage human embryo is considered sexually indifferent. Explain why this is so even though the embryo's genotypic sex is already fixed.

8. What specific observations (evidence) support the conclusions about sex determination in *Drosophila* and humans?

9. Describe how nondisjunction in human female gametes can give rise to Klinefelter and Turner syndrome offspring following fertilization by a normal male gamete.

10. An insect species is discovered in which the heterogametic sex is unknown. An X-linked recessive mutation for *reduced wing* (*rw*) is discovered. Contrast the F_1 and F_2 generations from a cross between a female with reduced wings and a male with normal-sized wings when

    (a) the female is the heterogametic sex.
    (b) the male is the heterogametic sex.

11. When cows have twin calves of unlike sex (fraternal twins), the female twin is usually sterile and has masculinized reproductive organs. This calf is referred to as a freemartin. In cows, twins may share a common placenta and thus fetal circulation. Predict why a freemartin develops.

12. An attached-X female fly, $\widehat{XX}Y$ (see the "Insights and Solutions" box), expresses the recessive X-linked *white*-eye mutation. It is crossed to a male fly that expresses the X-linked recessive *miniature*-wing mutation. Determine the outcome of this cross in terms of sex, eye color, and wing size of the offspring.

13. Assume that on rare occasions the attached X chromosomes in female gametes become unattached. Based on the parental phenotypes in Problem 12, what outcomes in the F_1 generation would indicate that this has occurred during female meiosis?

14. It has been suggested that any male-determining genes contained on the Y chromosome in humans cannot be located in the limited region that synapses with the X chromosome during meiosis. What might be the outcome if such genes were located in this region?

15. What is a Barr body, and where is it found in a cell?

16. Indicate the expected number of Barr bodies in interphase cells of individuals with Klinefelter syndrome; Turner syndrome; and karyotypes 47,XYY, 47,XXX, and 48,XXXX.

17. Define the Lyon hypothesis.

18. Can the Lyon hypothesis be tested in a human female who is homozygous for one allele of the X-linked *G6PD* gene? Why, or why not?

19. Predict the potential effect of the Lyon hypothesis on the retina of a human female heterozygous for the X-linked red-green color blindness trait.

20. Cat breeders are aware that kittens expressing the X-linked calico coat pattern and tortoiseshell pattern are almost invariably females. Why are they certain of this?

21. In mice, the *Sry* gene (see Section 7.2) is located on the Y chromosome very close to one of the pseudoautosomal regions that pairs with the X chromosome during male meiosis. Given this information, propose a model to explain the generation of unusual males who have two X chromosomes (with an *Sry*-containing piece of the Y chromosome attached to one X chromosome).

22. The genes encoding the red- and green-color-detecting proteins of the human eye are located next to one another on the X chromosome and probably evolved from a common ancestral pigment gene. The two proteins demonstrate 76 percent homology in their amino acid sequences. A normal-visioned woman (with both genes present on each of her two X chromosomes) has a red-color-blind son who was shown to have one copy of the green-detecting gene and no copies of the red-detecting gene. Devise an explanation for these observations at the chromosomal level (involving meiosis).

23. What is the role of the enzyme aromatase in sexual differentiation in reptiles?

Extra-Spicy Problems

24. In the wasp *Bracon hebetor*, a form of parthenogenesis (the development of unfertilized eggs into progeny) resulting in haploid organisms is not uncommon. All haploids are males. When offspring arise from fertilization, females almost invariably result. P. W. Whiting has shown that an X-linked gene with nine multiple alleles (X_a, X_b, etc.) controls sex determination. Any homozygous or hemizygous condition results in males, and any heterozygous condition results in females. If an X_a/X_b female mates with an X_a male and lays 50 percent fertilized and 50 percent unfertilized eggs, what proportion of male and female offspring will result?

25. The Amami spiny rat (*Tokudaia osimensis*) lacks a Y chromosome, yet scientists at Hokkaido University in Japan have reported that key sex-determining genes continue to be expressed in this species. Provide possible explanations for why male differentiation can still occur in this mammalian species despite the absence of a Y chromosome.

26. In mice, the X-linked dominant mutation *Testicular feminization (Tfm)* eliminates the normal response to the testicular hormone testosterone during sexual differentiation. An XY mouse bearing the *Tfm* allele on the X chromosome develops testes, but no further male differentiation occurs—the external genitalia of such an animal are female. From this information, what might you conclude about the role of the *Tfm* gene product and the X and Y chromosomes in sex determination and sexual differentiation in mammals? Can you devise an experiment, assuming you can "genetically engineer" the chromosomes of mice, to test and confirm your explanation?

27. When the cloned cat Carbon Copy (CC) was born (see the Now Solve This question on p. 161), she had black patches and white patches, but completely lacked any orange patches. The knowledgeable students of genetics were not surprised at this outcome. Starting with the somatic ovarian cell used as the source of the nucleus in the cloning process, explain how this outcome occurred.

28. In reptiles, sex determination was thought to be controlled by sex-chromosome systems or by temperature-dependent sex determination without an inherited component to sex. But as we discussed in section 7.6, in the Australian lizard, *Pogona vitticeps*, it was recently revealed that sex is determined by both chromosome composition and by the temperature at which eggs are incubated. What effects might climate change have on temperature-dependent sex determination in this species, and how might this impact the sex ratio for this species in subsequent generations?

29. In chickens, a key gene involved in sex determination has recently been identified. Called *DMRT1*, it is located on the Z chromosome and is absent on the W chromosome. Like *SRY* in humans, it is male determining. Unlike *SRY* in humans, however, female chickens (ZW) have a single copy while males (ZZ) have two copies of the gene. Nevertheless, it is transcribed only in the developing testis. Working in the laboratory of Andrew Sinclair (a co-discoverer of the human SRY gene), Craig Smith and colleagues were able to "knock down" expression of *DMRT1* in ZZ embryos using RNA interference techniques (see Chapter 18). In such cases, the developing gonads look more like ovaries than testes [*Nature* 461: 267 (2009)]. What conclusions can you draw about the role that the *DMRT1* gene plays in chickens in contrast to the role the *SRY* gene plays in humans?