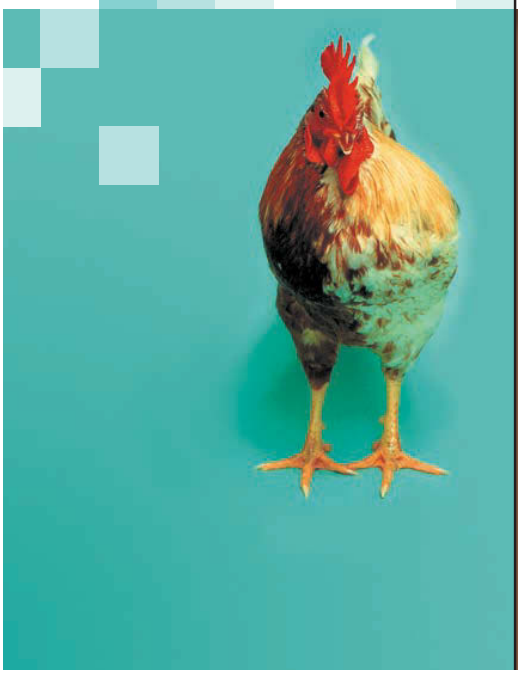
6

# Sex Determination and Gametogenesis

How can this chicken become half hen and half rooster?



**"SEXUAL REPRODUCTION IS … THE MASTERPIECE OF NATURE,"** wrote Erasmus Darwin in 1791. Male and female offspring are generated by equivalent, equally active, gene-directed processes, neither being "higher" or "lower" or "greater" or "lesser" than the other. In mammals and flies, the sex of the individual is determined when the gametes—sperm and egg—come together. As we will see, however, there are other schemes of sex determination where animals of certain species are both male and female (making both sperm and eggs), and schemes where the environment determines an individual's sex. The gametes are the product of a **germ line** that is separate from the somatic cell lineages that divide mitotically to generate the differentiated somatic cells of the developing individual. Cells in the germ line undergo meiosis, a remarkable process of cell division by which the chromosomal content of a cell is halved so that the union of two gametes in fertilization restores the full chromosomal complement of the new organism. Sexual reproduction means that each new organism receives genetic material from two distinct parents, and the mechanisms of meiosis provide an incredible amount of genomic variation upon which evolution can work.

Gametogenesis and fertilization are both the end and the beginning of the circle of life. This chapter describes how the sex of an individual organism is determined, which in turn will determine whether that individual's gametes will become sperm or eggs.

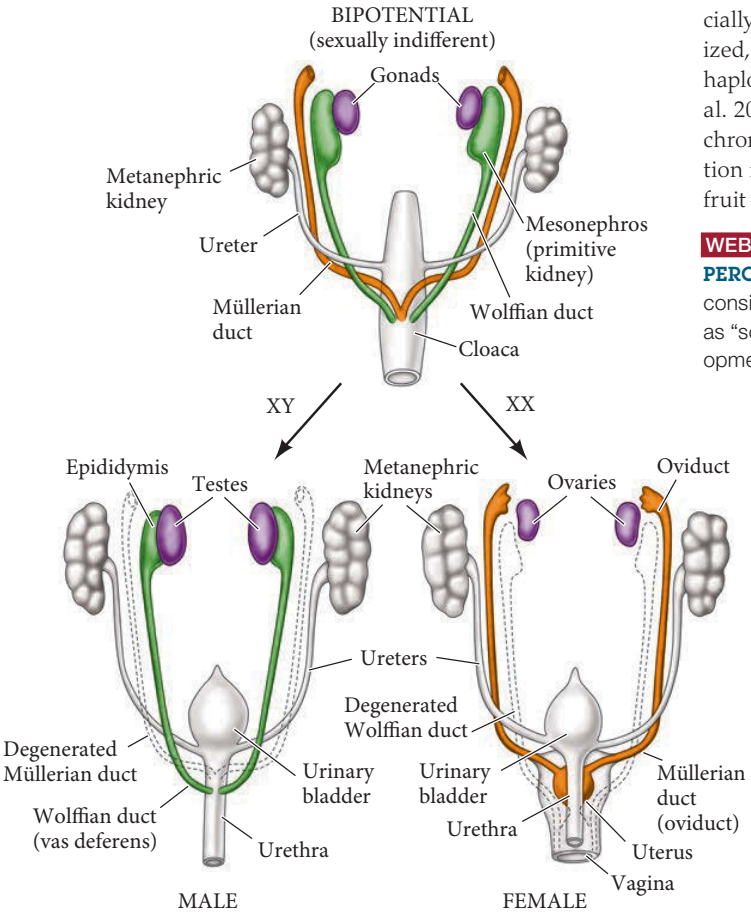
## The Punchline

In vertebrates and arthropods, sex is determined by chromosomes. In mammals, the *Sry* gene on the Y chromosome transforms the bipotential gonad into a testis (and prevents ovary development), while inheritance of two X chromosomes activates β-catenin, transforming the bipotential gonad into an ovary (and preventing testis formation). In flies, the number of X chromosomes regulates the *Sxl* gene, enabling differential splicing of particular nuclear RNAs into male- or female-specific mRNAs. In mammals, the testes secrete hormones such as testosterone and anti-Müllerian hormone. The first builds the male phenotype, the second blocks the female phenotype. The ovaries synthesize estrogen that builds the female phenotype; they also secrete progesterone to maintain pregnancy. In all species, the gonads instruct gametogenesis, the development of the germ cells. Mammalian germ cells entering the ovaries initiate meiosis while in the embryo and become oocytes. Germ cells entering the mammalian testes are prevented from entering meiosis and instead divide to produce a stem cell population that at puberty will generate the sperm. There are also animal species whose sex is determined by environmental factors such as temperature.

## Chromosomal Sex Determination

There there are several ways chromosomes can determine the sex of an embryo. In *mammals*, the presence of either a second X chromosome or a Y chromosome determines whether the embryo will be female (XX) or male (XY). In *birds*, the situation is reversed (Smith and Sinclair 2001): the male has the two similar sex chromosomes (ZZ) and the female has the unmatched pair (ZW). In *flies*, the Y chromosome plays no role in sex determination, but the number of X chromosomes appears to determine the sexual phenotype. In other insects (especially hymenopterans such as bees, wasps, and ants), fertilized, diploid eggs develop into females, while unfertilized, haploid eggs become males (Beukeboom 1995; Gempe et al. 2009). This chapter will discuss only two of the many chromosomal modes of sex determination: sex determination in placental mammals and sex determination in the fruit fly *Drosophila*.

**WEB TOPIC 6.1 SEX DETERMINATION AND SOCIAL PERCEPTIONS** In the not-so-distant past, femaleness was considered a "default state," while maleness was thought of as "something more," acquired by genes that propelled development farther.




## The Mammalian Pattern of Sex Determination

Mammalian sex determination is governed by the gonad-forming genes and by the hormones elaborated by the gonads. **Primary sex determination** is *the determination of the gonads*—the egg-forming ovaries or sperm-forming testes. **Secondary sex determination** is *the determination of the male or female phenotype by the hormones produced by the gonads*. The formation both of ovaries and of testes is an active, gene-directed process. Both the male and female gonads diverge from a common precursor, the **bipotential gonad** (sometimes called the **indifferent gonad**) (**FIGURE 6.1**).

| | | |
|---|---|---|
| GONADS | | |
| Gonadal type | Testis | Ovary |
| Germ cell location | Inside testis cords (in medulla of testis) | Inside follicles of ovarian cortex |
| DUCTS | | |
| Remaining duct | Wolffian | Müllerian |
| Duct differentiation | Vas deferens, epididymis, seminal vesicle | Oviduct, uterus, cervix, upper portion of vagina |
| UROGENITAL SINUS | Prostate | Skene's glands |
| LABIOSCROTAL FOLDS | Scrotum | Labia majora |
| GENITAL TUBERCLE | Penis | Clitoris |

**FIGURE 6.1** Development of gonads and their ducts in mammals. Originally, a bipotential (indifferent) gonad develops, with undifferentiated Müllerian ducts (female) and Wolffian ducts (male) ducts both present. If XY, the gonads becomes testes and the Wolffian duct persists. If XX, the gonads become ovaries and the Müllerian duct persists. Hormones from the gonads will cause the external genitalia to develop either in the male direction (penis, scrotum) or the female direction (clitoris, labia majora).

In mammals, primary sex determination is dictated by whether an organism has an XX or an XY karyotype. In most cases, the female's karyotype is XX and the male's is XY. Every individual must carry at least one X chromosome. Since the diploid female is XX, each of her haploid eggs has a single X chromosome. The male, being XY, generates two populations of haploid sperm: half will bear an X chromosome, half a Y. If at fertilization the egg receives a second X chromosome from the sperm, the resulting individual is XX, forms ovaries, and is female; if the egg receives a Y chromosome from the sperm, the individual is XY, forms testes, and is male (**FIGURE 6.2A**; Stevens 1905; Wilson 1905; see Gilbert 1978).

The Y chromosome carries a gene that encodes a **testis-determining factor** that organizes the bipotential gonad into a testis. This was demonstrated in 1959 when karyotyping showed that XXY individuals (a condition known as Klinefelter syndrome) are male (despite having two X chromosomes), and that individuals having only one X chromosome (XO, sometimes called Turner syndrome) are female (Ford et al. 1959;

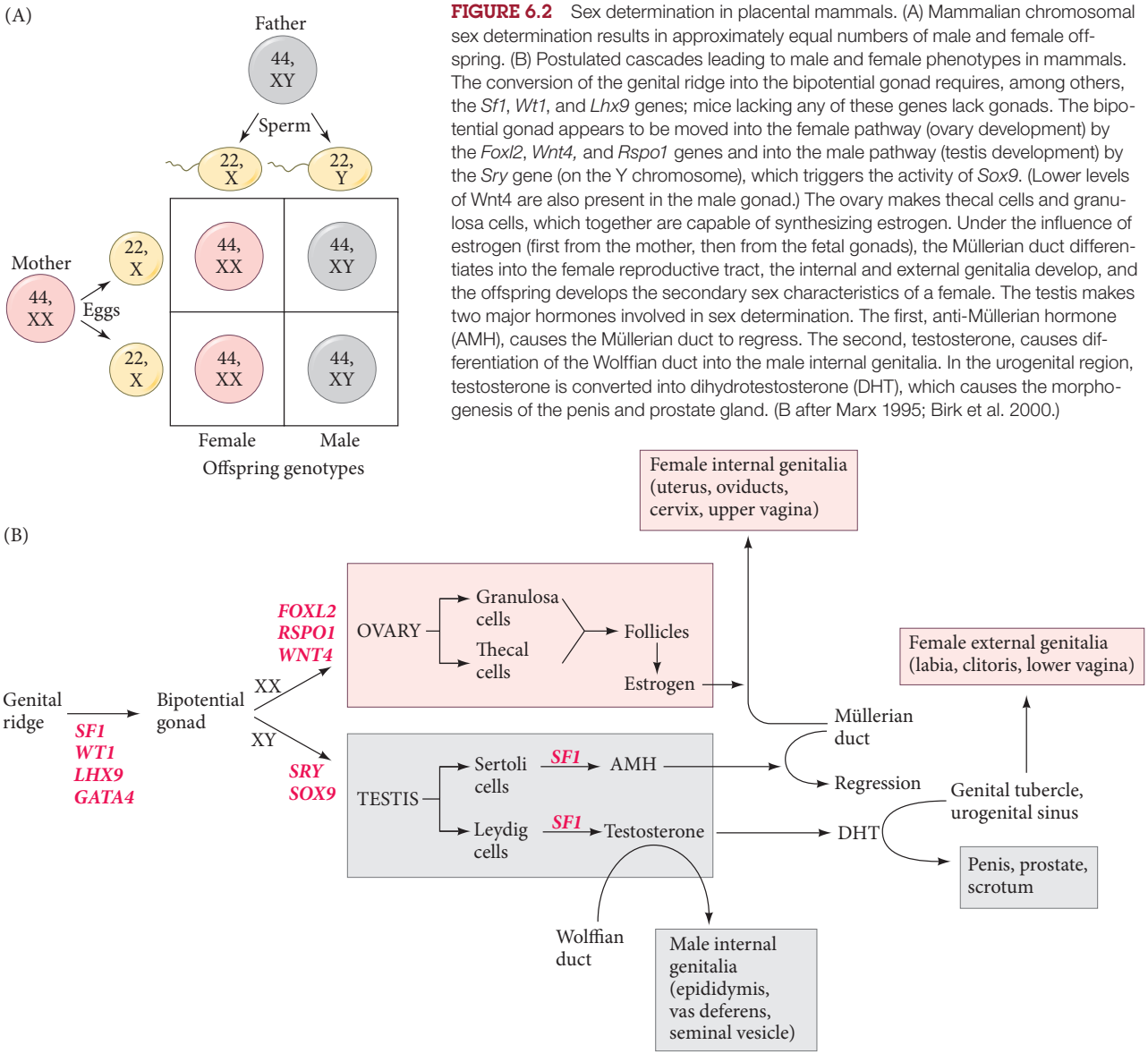



**FIGURE 6.2** Sex determination in placental mammals. (A) Mammalian chromosomal sex determination results in approximately equal numbers of male and female offspring. (B) Postulated cascades leading to male and female phenotypes in mammals. The conversion of the genital ridge into the bipotential gonad requires, among others, the *Sf1*, *Wt1*, and *Lhx9* genes; mice lacking any of these genes lack gonads. The bipotential gonad appears to be moved into the female pathway (ovary development) by the *Foxl2*, *Wnt4*, and *Rspo1* genes and into the male pathway (testis development) by the *Sry* gene (on the Y chromosome), which triggers the activity of *Sox9*. (Lower levels of Wnt4 are also present in the male gonad.) The ovary makes thecal cells and granulosa cells, which together are capable of synthesizing estrogen. Under the influence of estrogen (first from the mother, then from the fetal gonads), the Müllerian duct differentiates into the female reproductive tract, the internal and external genitalia develop, and the offspring develops the secondary sex characteristics of a female. The testis makes two major hormones involved in sex determination. The first, anti-Müllerian hormone (AMH), causes the Müllerian duct to regress. The second, testosterone, causes differentiation of the Wolffian duct into the male internal genitalia. In the urogenital region, testosterone is converted into dihydrotestosterone (DHT), which causes the morphogenesis of the penis and prostate gland. (B after Marx 1995; Birk et al. 2000.)

Jacobs and Strong 1959). XXY men have functioning testes. Women with a single X chromosome begin making ovaries, but the ovarian follicles cannot be maintained without the second X chromosome. Thus, a second X chromosome completes the ovaries, whereas the presence of a Y chromosome (even when multiple X chromosomes are present) initiates the development of testes.

The reason the Y chromosome is able to direct testis formation even when more than one X chromosome is present may be a matter of timing. It appears there is a crucial window of opportunity during gonad development during which the testis-determining factor (now known to be the product of the *Sry* gene) can function. If the *Sry* gene is present, it usually acts during this duration to promote testis formation and to inhibit ovary formation. If the *Sry* gene is not present (or if it fails to act at the appropriate time), the ovary-forming genes are the ones that will function (**FIGURE 6.2B**; Hiramatsu et al. 2009; Kashimada and Koopman 2010).

Once primary (chromosomal) determination has established the gonads, the gonads begin to produce the hormones and paracrine factors that govern secondary sex determination—development of the sexual phenotype outside the gonads. This includes the male or female duct systems and the external genitalia. A male mammal has a penis, scrotum (testicle sac), seminal vesicles, and prostate gland. A female mammal has a uterus, oviducts, cervix, vagina, clitoris, labia, and mammary glands.[1] In many species, each sex also has a sex-specific body size, vocal cartilage, and musculature. Secondary sex characteristics are usually determined by hormones and paracrine factors secreted from the gonads. In the absence of gonads, it appears the female phenotype is generated. When Jost (1947, 1953) removed fetal rabbit gonads before they had differentiated, the resulting rabbits had a female phenotype, regardless of whether their genotype was XX or XY.

The general scheme of primary sex determination is shown in Figure 6.2B. If the embryonic cells have two X chromosomes and no Y chromosome, the gonadal primordia develop into ovaries. The ovaries produce **estrogen**, a hormone that enables the development of the **Müllerian duct** into the uterus, oviducts, cervix, and upper portion of the vagina (Fisher et al. 1998; Couse et al. 1999; Couse and Korach 2001). If embryonic cells contain both an X and a Y chromosome, testes form and secrete two major factors. The first is a TGF-β family paracrine factor called **anti-Müllerian hormone** (**AMH**; sometimes called **Müllerian-inhibiting factor**, **MIF**). AMH destroys the Müllerian duct, thus preventing formation of the uterus and oviducts. The second factor is the steroid hormone **testosterone**. Testosterone masculinizes the fetus, stimulating formation of the penis, male duct system, scrotum, and other portions of the male anatomy, as well as inhibiting development of the breast primordia.

**DEV TUTORIAL** ***Mammalian sex determination*** Scott Gilbert outlines the sex determination schemes of mammals.

## Primary Sex Determination in Mammals

Mammalian gonads embody a unique embryological situation. All other organ rudiments normally can differentiate into only one type of organ—a lung rudiment can only become a lung, a liver rudiment can develop only into a liver. The gonadal rudiment, however, has two options: it can develop into either an ovary or a testis, two organs with very different tissue architectures. The path of differentiation taken by the gonadal rudiment is dictated by the genotype and determines the future sexual development of the organism (Lillie 1917). But before this decision is made, the mammalian gonad first develops through a bipotential, or indifferent, stage during which it has neither female nor male characteristics (see Figure 6.1).

[1] The naturalist Carolus Linnaeus named the mammals after this female secondary sexual trait in the seventeenth century. The politics of this decision is discussed in Schiebinger 1993.

### *The developing gonads*

In humans, two gonadal rudiments appear during week 4 and remain sexually indifferent until week 7. These gonadal precursors are paired regions of the mesoderm adjacent to the developing kidneys (Tanaka and Nishinakamura 2014; **FIGURE 6.3A,B**). The **germ cells**—the precursors of either sperm or eggs—migrate into the gonads during week 6 and are surrounded by the mesodermal cells.

*If the fetus is XY,* the mesodermal cells continue to proliferate through week 8, when a subset of these cells initiate their differentiation into **Sertoli cells**. During embryonic development, the developing Sertoli cells secrete the anti-Müllerian hormone that blocks development of the female ducts. These same Sertoli epithelial cells will also form the seminiferous tubules that will support the development of sperm throughout the lifetime of the male mammal.

During week 8, the developing Sertoli cells surround the incoming germ cells and organize themselves into the **testis cords**. These cords form loops in the central region of the developing testis and are connected to a network of thin canals, called the **rete testis**, located near the developing kidney duct (**FIGURE 6.3C,D**). Thus, when germ cells enter the male gonads, they will develop within the testis cords, *inside* the organ. Later in development (at puberty in humans; shortly after birth in mice, which procreate much faster), the testis cords mature to form the **seminiferous tubules**. The germ cells migrate to the periphery of these tubules, where they establish the spermatogonial stem cell population that produces sperm throughout the lifetime of the male (see Figure 6.21). In the mature seminiferous tubule, sperm are transported from the inside of the testis through the rete testis, which joins the **efferent ducts**. The efferent ducts are the remodeled tubules of the developing kidney. During male development, the Wolffian duct differentiates to become the **epididymis** (adjacent to the testis) and the **vas deferens** (the tube through which sperm pass into the urethra and out of the body). Note that both sperm and urine will use the urethra to exit the body.

Meanwhile, the other group of mesoderm cells (those that did not form the Sertoli epithelium) differentiate into a mesenchymal cell type, the testosterone-secreting **Leydig cells**. Thus, the fully developed testis will have epithelial tubes made of Sertoli cells that enclose the germ cells, as well as a mesenchymal cell population, the Leydig cells, that secrete testosterone. Each incipient testis is surrounded by a thick extracellular matrix, the tunica albuginea, which helps protect it.

*If the fetus is XX,* the sex cords in the center of the developing gonad degenerate, leaving sex cords at the surface (cortex) of the gonad. Each germ cell gets enveloped by a separate cluster of sex cord epithelial cells (**FIGURE 6.3E,F**). The germ cells will become **ova** (eggs), and the surrounding cortical epithelial cells will differentiate into **granulosa cells**. The remaining mesenchyme cells of the developing ovary differentiate into **thecal cells**. Together, the thecal and granulosa cells form **follicles** that envelop the germ cells and secrete steroid hormones such as estrogens and (when pregnant) progesterone. Each follicle contains a single germ cell—an **oogonium** (egg precursor)—which will enter meiosis at this time.

There is a reciprocal relationship between the germ cells and the somatic cells of the gonads. The germ cells are originally bipotential and can become either sperm or eggs. Once in the male or female sex cords, however, they are instructed to either (1) begin meiosis and become eggs, or (2) remain meiotically dormant and become spermatogonia (McLaren 1995; Brennan and Capel 2004). In XX gonads, germ cells are essential for the maintenance of ovarian follicles. Without germ cells, the follicles degenerate into cordlike structures and express male-specific markers. In XY gonads, the germ cells help support the differentiation of Sertoli cells, although testis cords will form even without the germ cells, albeit a bit later (McLaren 1991). When an ovary is being formed, the Müllerian duct remains intact (there is no AMH to destroy it), and it differentiates into the oviducts, uterus, cervix, and upper vagina. In the absence of adequate testosterone, the Wolffian duct degenerates (see Figures 6.1 and 6.2).

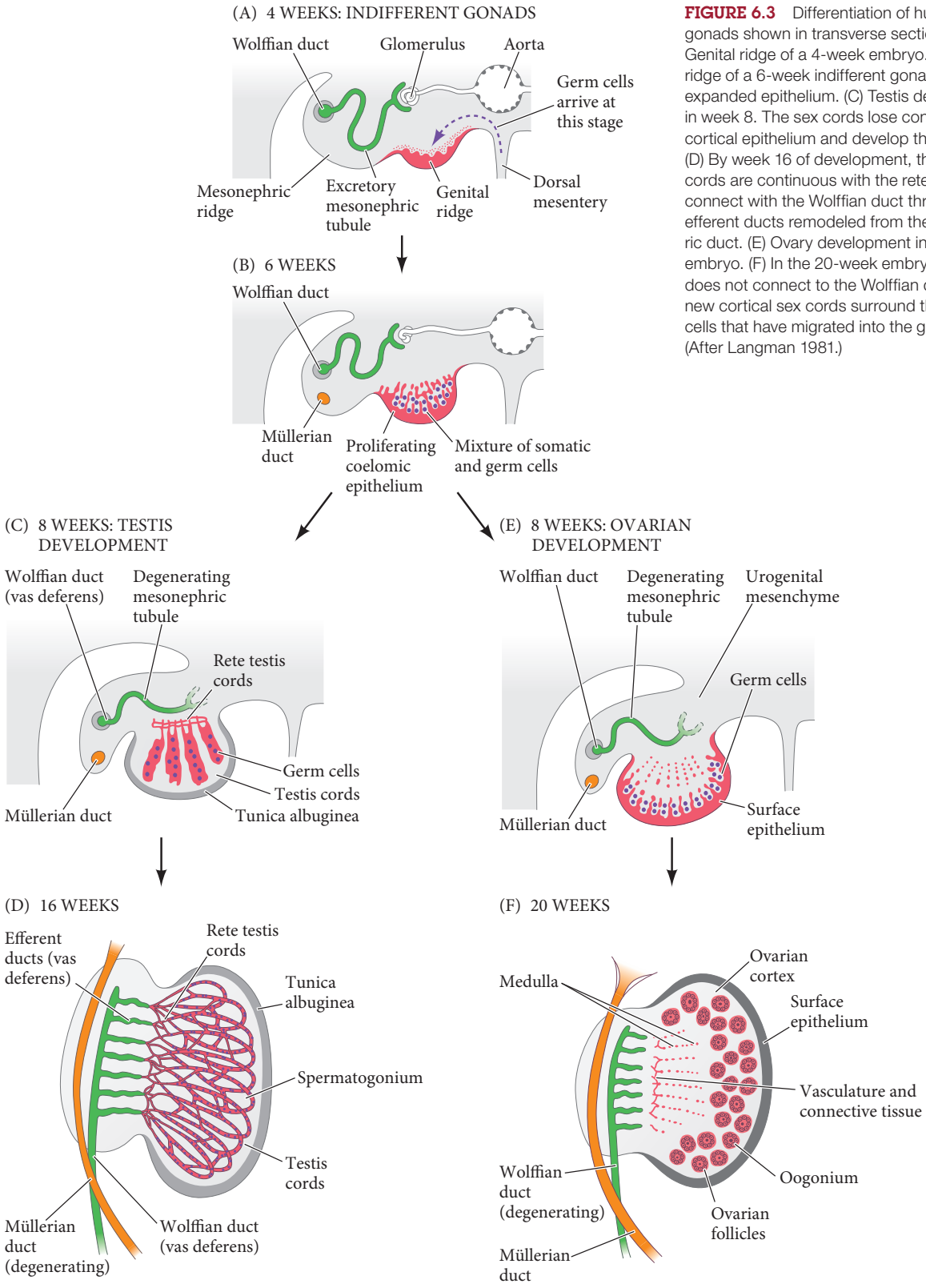



**FIGURE 6.3** Differentiation of human gonads shown in transverse section. (A) Genital ridge of a 4-week embryo. (B) Genital ridge of a 6-week indifferent gonad showing expanded epithelium. (C) Testis development in week 8. The sex cords lose contact with the cortical epithelium and develop the rete testis. (D) By week 16 of development, the testis cords are continuous with the rete testis and connect with the Wolffian duct through the efferent ducts remodeled from the mesonephric duct. (E) Ovary development in an 8-week embryo. (F) In the 20-week embryo, the ovary does not connect to the Wolffian duct, and new cortical sex cords surround the germ cells that have migrated into the genital ridge. (After Langman 1981.)

### *Genetic mechanisms of primary sex determination: Making decisions*

Several human genes have been identified whose function is necessary for normal sexual differentiation. Because the phenotype of mutations in sex-determining genes is often sterility, clinical infertility studies have been useful in identifying those genes that are active in determining whether humans become male or female. Experimental manipulations to confirm the functions of these genes can then be done in mice.

The story starts in the bipotential gonad that has not yet been committed to the male or female direction. The genes for transcription factors Wt1, Lhx9, GATA4, and Sf1 are expressed, and the loss of function of any one of them will prevent the normal development of either male or female gonads. Then the decision is made:

- *If no Y chromosome is present,* these transcription and paracrine factors are thought to activate further expression of Wnt4 protein (already expressed at low levels in the genital epithelium) and of a small soluble protein called R-spondin1 (Rspo1). Rspo1 binds to its cell membrane receptor and further stimulates the Disheveled protein of the Wnt pathway, making the Wnt pathway more efficient at producing the transcriptional regulator β-catenin. One of the several functions of β-catenin in gonadal cells is to further activate the genes for Rspo1 and Wnt4, creating a positive feedback loop between these two proteins. A second role of β-catenin is to initiate the ovarian pathway of development by activating those genes involved in granulosa cell differentiation. Its third role is to prevent the production of Sox9, a protein crucial for testis determination (Maatouk et al. 2008; Bernard et al. 2012).
- *If a Y chromosome is present,* the same set of factors in the bipotential gonad activates the *Sry* gene on the Y chromosome. Sry protein binds to the enhancer of the *Sox9* gene and elevates expression of this key gene in the testis-determining pathway (Bradford et al. 2009b; Sekido and Lovell-Badge 2009). Sox9 and Sry also act to block the ovary-forming pathway, possibly by blocking β-catenin (Bernard et al. 2008; Lau and Li 2009).

**FIGURE 6.4** shows one possible model of how primary sex determination can be initiated. Here we see an important rule of animal development: a pathway for cell specification often has two components, with one branch that says "Make A" and another branch that says "… and *don't* make B." In the case of the gonads, the male pathway says "Make testes and don't make ovaries," while the female pathway says "Make ovaries and don't make testes."

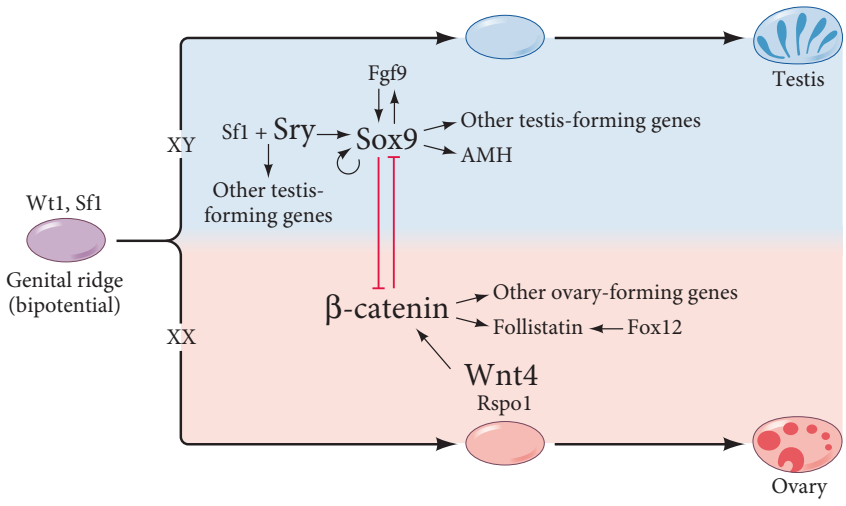



**FIGURE 6.4** Possible mechanism for the initiation of primary sex determination in mammals. While we do not know the specific interactions involved, this model attempts to organize the data into a coherent sequence. If Sry is *not present* (pink region), the interactions between paracrine and transcription factors in the developing genital ridge activate *Wnt4* and *Rspo1*. Wnt4 activates the canonical Wnt pathway, which is made more efficient by Rspo1. The Wnt pathway causes the accumulation of β-catenin, and large accumulation of β-catenin stimulates further Wnt4 activity. This continual production of β-catenin both induces the transcription of ovary-producing genes and blocks the testis-determining pathway by interfering with *Sox9* activity. If Sry is *present* (blue region), it may block β-catenin signaling (thus halting ovary generation) and, along with Sf1, activate the *Sox9* gene. Sox9 activates Fgf9 synthesis, which stimulates testis development and promotes further Sox9 synthesis. Sox9 also prevents β-catenin's activation of ovary-producing genes. Sry may also activate other genes (such as *TCF21* and *NT3*) that help generate Sertoli cells. In summary, then, a Wnt4/β-catenin loop specifies the ovaries, whereas a Sox9/Fgf9 loop specifies the testes. One of the targets of the Wnt pathway is the *follistatin* gene, whose product organizes the granulosa cells of the ovary. Transcription factor Foxl2, which is activated (in a still unknown way) in the ovary, is also involved in inducing follistatin synthesis. The XY pathway appears to have an earlier initiation; if it does not function, the XX pathway takes over. (After Sekido and Lovell-Badge 2009; McClelland et al. 2012.)

### *The ovary pathway: Wnt4 and R-spondin1*

In mice, the paracrine factor **Wnt4** is expressed in the bipotential gonads, but its expression becomes undetectable in XY gonads as they become testes, whereas it is maintained in XX gonads as they begin to form ovaries. In XX mice that lack the *Wnt4* gene, the ovary fails to form properly, and the cells transiently express testis-specific markers, including Sox9, testosterone-producing enzymes, and AMH (Vainio et al. 1999; Heikkilä et al. 2005). Thus, Wnt4 appears to be an important factor in ovary formation, although it is not the only determining factor.

**R-spondin1** (**Rspo1**) is also critical in ovary formation, since in human case studies, several XX individuals with *RSPO1* gene mutations became phenotypic males (Parma et al. 2006). Rspo1 acts in synergy with Wnt4 to produce β-catenin, which appears to be critical both in activating further ovarian development and in blocking the synthesis of a testis-determining factor, Sox9 (Maatouk et al. 2008; Jameson et al. 2012). In XY individuals with a duplication of the region on chromosome 1 that contains both the *WNT4* and *RSPO1* genes, the pathways that make β-catenin override the male pathway, resulting in a male-to-female sex reversal. Similarly, if XY mice are induced to overexpress β-catenin in their gonadal rudiments, they form ovaries rather than testes. Indeed, β-catenin appears to be a key "pro-ovarian/anti-testis" signaling molecule in all vertebrate groups, as it is seen in the female (but not the male) gonads of birds, mammals, and turtles. These three groups have very different modes of sex determination, yet Rspo1 and β-catenin are made in the ovaries of each of them (**FIGURE 6.5**; Maatouk et al. 2008; Cool and Capel 2009; Smith et al. 2009).

Certain transcription factors whose genes are activated by β-catenin are found exclusively in the ovaries. One possible target for β-catenin is the gene encoding TAFII-105 (Freiman et al. 2002). This transcription factor subunit (which helps bind RNA polymerase to promoters) is seen only in ovarian follicle cells. Female mice lacking this subunit have small ovaries with few, if any, mature follicles. The transcription factor Foxl2 is another protein that is strongly upregulated in ovaries, and XX mice homozygous for mutant *Foxl2* alleles develop male-like gonad structure and upregulate *Sox9* gene expression and testosterone production. Both Foxl2 and β-catenin are critical for activation of the *Follistatin* gene (Ottolenghi et al. 2005; Kashimada et al. 2011; Pisarska et al. 2011). Follistatin, an inhibitor of TGF-β family of paracrine factors, is thought to be the protein responsible for organizing the epithelium into the granulosa cells of the ovary (Yao et al. 2004). XX mice lacking follistatin in the developing gonad undergo a partial sex reversal, forming testicle-like structures. Numerous other transcription factors are upregulated by the Wnt4/R-spondin signal (Naillat et al. 2015), and we are just beginning to figure out how the components of the ovary-forming pathway are integrated.

As important as the *construction* of the ovaries is, the *maintenance* of the ovarian structure is also critical. Similarly, the maintenance of testicular phenotype is as critical as its original construction. Remarkably, gonadal organization is not stable throughout life, and without proper gene expression, female follicles can become male tubules and male tubules can become female follicles. In females, the maintainer of ovarian identity appears to be Foxl2

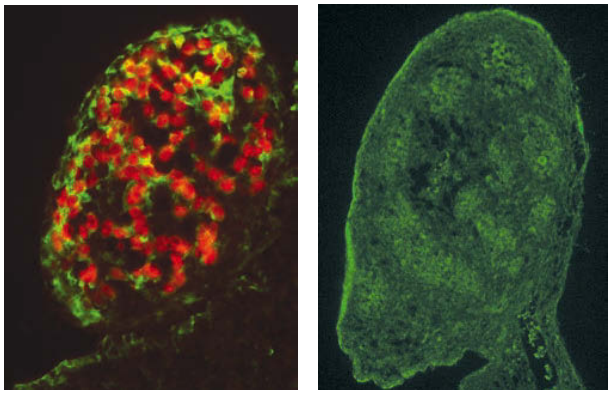



**FIGURE 6.5** Localization of Rspo1 protein in embryonic day 14.5 mouse gonads. Immunofluorescent probes were used to identify Rspo1 (green) and the meiotic germ cell marker, Scp3 (red). (A) Rspo1 was found on somatic cells and at the germ cell surface of the ovaries. (B) These antibodies revealed neither Rspo1 nor Scp3 in the developing testes. (The germ cells in the male gonads have not entered meiosis at this point in development, whereas the ovarian germ cells have.) (From Smith et al. 2008; photograph courtesy of C. Smith.)

(Uhlenhaut et al. 2009). When Foxl2 is deleted in adult-stage ovaries, the Sox9 gene becomes active and the ovary is transformed into a testis.

### *The testis pathway: Sry and Sox9*

***SRY*: THE Y CHROMOSOME SEX DETERMINANT** In humans, the major gene for testis determination resides on the short arm of the Y chromosome. By analyzing the DNA of rare XX men and XY women (i.e., individuals who are genotypically one sex but phenotypically the other), the position of the testis-determining gene was narrowed down to a 35,000-base-pair region of the Y chromosome found near the tip of the short arm. In this region, Sinclair and colleagues (1990) found a male-specific DNA sequence that encodes a peptide of 223 amino acids. This gene is called ***Sry*** (**sex-determining region of the *Y* chromosome**), and there is extensive evidence that it is indeed the gene that encodes the human testis-determining factor.

*Sry* is found in normal XY males and also in the rare XX males; it is absent from normal XX females and from many XY females. Approximately 15% of human XY females have the *SRY* gene, but their copies of the gene contain point or frameshift mutations that prevent Sry protein from binding to DNA (Pontiggia et al. 1994; Werner et al. 1995). If the *SRY* gene actually does encode the major testis-determining factor, one would expect it to act in the indifferent gonad immediately before or during testis differentiation. This prediction has been found to be the case in studies of the homologous gene in mice. The mouse *Sry* gene also correlates with the presence of testes; it is present in XX males and absent in XY females (Gubbay et al. 1990). *Sry* is expressed in the somatic cells of the bipotential gonads of XY mice immediately before the differentiation of these cells into Sertoli cells; its expression then disappears (Koopman et al. 1990; Hacker et al. 1995; Sekido et al. 2004).

The most impressive evidence for *Sry* being the gene for testis-determining factor comes from transgenic mice. If *Sry* induces testis formation, then inserting *Sry* DNA into the genome of a normal XX mouse zygote should cause that XX mouse to form testes. Koopman and colleagues (1991) took the 14-kilobase region of DNA that includes the *Sry* gene (and presumably its regulatory elements) and microinjected this sequence into the pronuclei of newly fertilized mouse zygotes. In several instances, XX embryos injected with this sequence developed testes, male accessory organs, and a penis[2] (**FIGURE 6.6**). Therefore, we conclude that *Sry*/*SRY* is the major gene on the Y chromosome for testis determination in mammals.

**SCIENTISTS SPEAK 6.1** Dr. Robin Lovell-Badge discusses his research showing how the *SRY* gene promotes testis formation in humans.

[2]These embryos did not form functional sperm—but they were not expected to. The presence of two X chromosomes prevents sperm formation in XXY mice and men, and the transgenic mice lacked the rest of the Y chromosome, which contains genes needed for spermatogenesis.

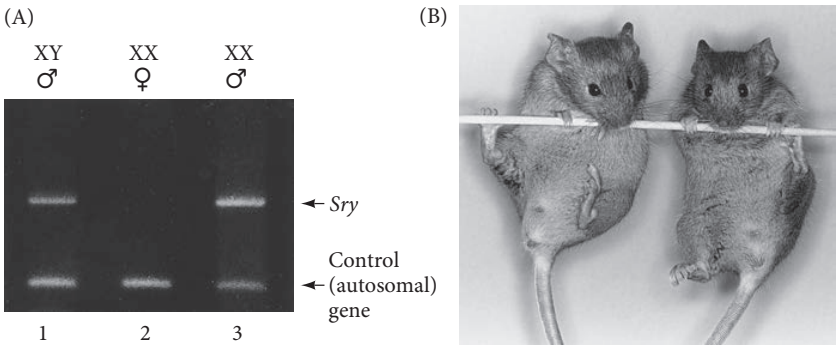
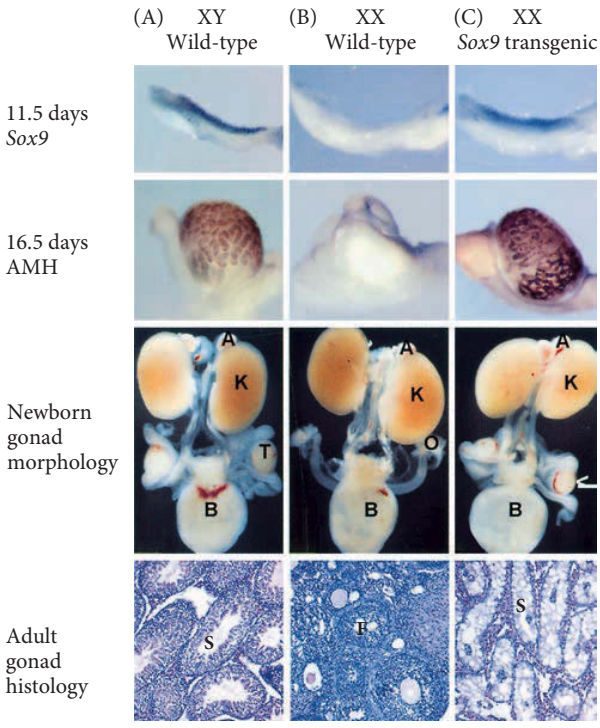



**FIGURE 6.6** An XX mouse transgenic for *Sry* is male. (A) Polymerase chain reaction followed by electrophoresis shows the presence of the *Sry* gene in normal XY males and in a transgenic XX/*Sry* mouse. The gene is absent in a female XX littermate. (B) The external genitalia of the transgenic mouse are male (right) and are essentially the same as those in an XY male (left). (From Koopman et al. 1991; photographs courtesy of the authors.)

***SOX9*: AN AUTOSOMAL TESTIS-DETERMINING GENE** For all its importance in sex determination, the *Sry* gene is probably active for only a few hours during gonadal development in mice. During this time, it synthesizes the Sry transcription factor, whose primary role appears to be to activate the ***Sox9*** gene (Sekido and Lovell-Badge 2008; for other targets of Sry, see Web Topic 6.2). *Sox9* is an autosomal gene involved in several developmental processes, most notably bone formation. In the gonadal rudiments, however, *Sox9* induces testis formation. XX humans who have an extra activated copy of *SOX9* develop as males even if they have no *SRY* gene, and XX mice transgenic for *Sox9* develop testes (**FIGURE 6.7A–D**; Huang et al. 1999; Qin and Bishop 2005). Knocking out the *Sox9* gene in the gonads of XY mice causes complete sex reversal (Barrionuevo et al. 2006). Thus, even if *Sry* is present, mouse gonads cannot form testes if *Sox9* is absent, so it appears that *Sox9* can replace *Sry* in testis formation. This is not altogether surprising; although the *Sry* gene is found specifically in mammals, *Sox9* is found throughout the vertebrate phyla.

Indeed, *Sox9* appears to be the older and more central sex determination gene in vertebrates (Pask and Graves 1999). In mammals, it is activated by Sry protein; in birds, frogs, and fish, it appears to be activated by the dosage of the transcription factor Dmrt1; and in those vertebrates with temperature-dependent sex determination, it is often activated (directly or indirectly) by the male-producing temperature. Expression of the *Sox9* gene is specifically upregulated by the combined expression of Sry and Sf1 proteins in Sertoli cell precursors (**FIGURE 6.7E–H**; Sekido et al. 2004; Sekido and Lovell-Badge 2008). Thus, Sry may act merely



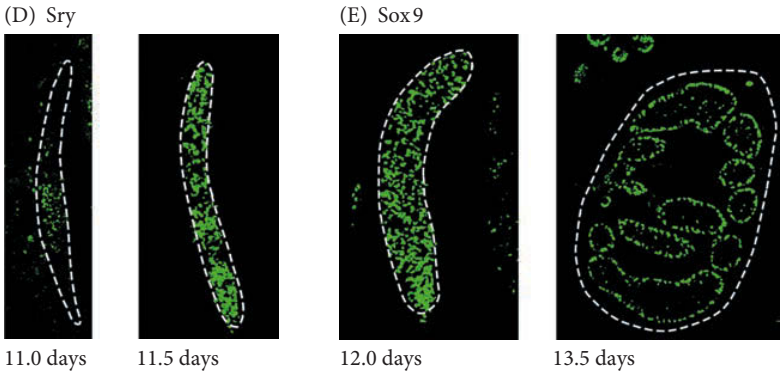




**FIGURE 6.7** Ability of Sox9 protein to generate testes. (A) A wild-type XY mouse embryo expresses the *Sox9* gene in the genital ridge at 11.5 days postconception, anti-Müllerian hormone in the embryonic gonad Sertoli cells at 16.5 days, and eventually forms descended testes with seminiferous tubules. K, kidneys; A, adrenal glands; B, bladder; T, testis; O, ovary; S, seminiferous tubule; F, follicle cell. (B) The wild-type XX embryo shows neither *Sox9* expression nor AMH. It constructs ovaries with mature follicle cells. (C) An XX embryo with the *Sox9* transgene inserted expresses *Sox9* and has AMH in 16.5-day Sertoli cells. It has descended testes, but the seminiferous tubules lack sperm (due to the presence of two X chromosomes in the Sertoli cells). (D,E) Chronological sequence from the expression of *Sry* in the genital ridge to that of *Sox9* in the Sertoli cells. (D) *Sry* expression. At day 11.0, Sry protein (green) is seen in the center of the genital ridge. At day 11.5, the domain of *Sry* expression increases and *Sox9* expression is activated. (E) By day 12.0, Sox9 protein (green) is seen in the same cells that earlier expressed *Sry*. By day 13.5, Sox9 is seen in those cells of the testis tubule that will become Sertoli cells. (A–C from Vidal et al. 2001, photographs courtesy of A. Schedl; D,E from Kashimada and Koopman, 2010, courtesy of P. Koopman.)

as a "switch" operating during a very short time to activate *Sox9*, and the Sox9 protein may initiate the conserved evolutionary pathway to testis formation. So, borrowing Eric Idle's phrase, Sekido and Lovell-Badge (2009) propose that Sry initiates testis formation by "a wink and a nudge."

The Sox9 protein has several functions. First, it appears to be able to activate its own promoter, thereby allowing it to be transcribed for long periods of time. Second, it blocks the ability of β-catenin to induce ovary formation, either directly or indirectly (Wilhelm et al. 2009). Third, it binds to *cis*-regulatory regions of numerous genes necessary for testis production (Bradford et al. 2009a). Fourth, Sox9 binds to the promoter site on the gene for anti-Müllerian hormone, providing a critical link in the pathway toward a male phenotype (Arango et al. 1999; de Santa Barbara et al. 2000). Fifth, Sox9 promotes the expression of the gene encoding Fgf9, a paracrine factor critical for testis development. Fgf9 is also essential for maintaining *Sox9* gene transcription, thereby establishing a positive feedback loop driving the male pathway (Kim et al. 2007).

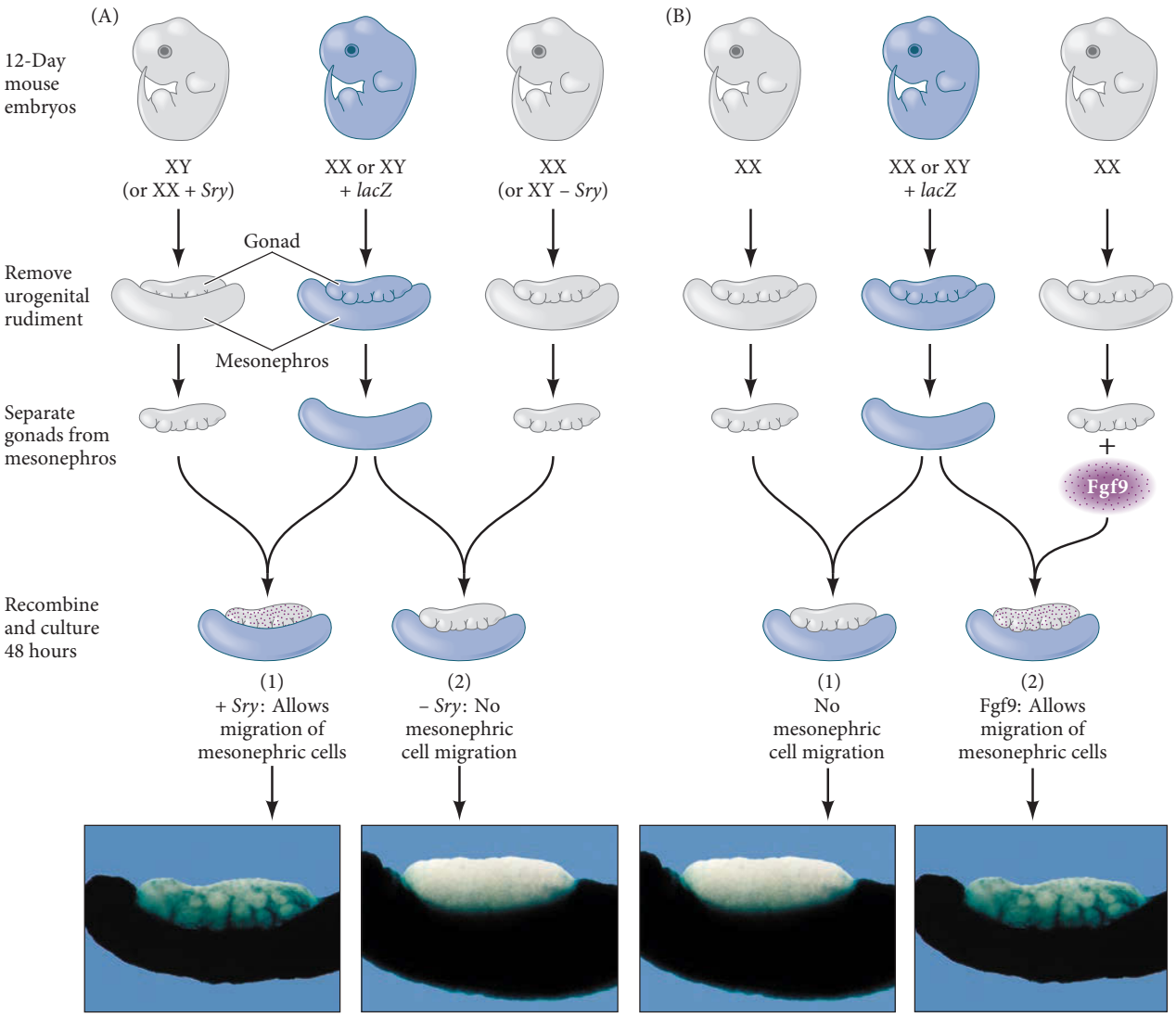
**WEB TOPIC 6.2** **FINDING THE ELUSIVE TESTIS-DETERMINING FACTOR** As one editor wrote, "The search for TDF has been a long and hard one."

**FIBROBLAST GROWTH FACTOR 9** When the gene for **fibroblast growth factor 9** (**Fgf9**) is knocked out in mice, the homozygous mutants are almost all female. Fgf9 protein, whose expression is dependent on Sox9 (Capel et al. 1999; Colvin et al. 2001), plays several roles in testis formation:

1. Fgf9 causes proliferation of the Sertoli cell precursors and stimulates their differentiation (Schmahl et al. 2004; Willerton et al. 2004).
2. It activates the migration of blood vessel cells from the adjacent kidney duct into the XY gonad. While this is normally a male-specific process, incubating XX gonads in Fgf9 leads to the migration of endothelial cells into XX gonads (**FIGURE 6.8**). These blood vessel cells form the major artery of the testis and play an instructive role in inducing the Sertoli cell precursors to form the testis cords; in their absence, testis cords do not form (Brennan et al. 2002; Combes et al. 2009).
3. It is required for maintaining *Sox9* expression in the presumptive Sertoli cells and directs their formation into tubules. Moreover, since it can act as both an autocrine and a paracrine factor, Fgf9 may coordinate Sertoli cell development by reinforcing *Sox9* expression in all the cells of the tissue (Hiramatsu et al. 2009). Such a "community effect" may be important in achieving the integrated assembly of testis tubules (Palmer and Burgoyne 1991; Cool and Capel 2009).
4. It represses Wnt4 signaling, which would otherwise direct ovarian development (Maatouk et al. 2008; Jameson et al. 2012).
5. Finally, Fgf9 appears to help coordinate the sex determination of the gonad with that of the germ cells. As we will see later in this chapter, those mammalian germ cells destined to become eggs enter meiosis quickly upon entering the gonad, whereas germ cells destined to become sperm delay their entry into meiosis until puberty. Fgf9 is one of the factors that blocks the immediate entry of germ cells into meiosis, thereby placing them onto the sperm-forming pathway (Barrios et al. 2010; Bowles et al. 2010).

**SF1: A CRITICAL LINK BETWEEN SRY AND THE MALE DEVELOPMENTAL PATHWAYS**

The transcription factor **steroidogenic factor 1** (**Sf1**) is necessary to make the bipotential gonad. But whereas Sf1 levels decline in the genital ridge of XX mouse embryos, they remain high in the developing testis. It is thought that Sry either directly or indirectly maintains *Sf1* gene expression. Sf1 protein appears to be active in masculinizing both the Leydig and the Sertoli cells. In the Sertoli cells, Sf1 works in collaboration with Sry to activate *Sox9* (Sekido and Lovell-Badge 2008) and then, working with Sox9, elevates levels of anti-Müllerian hormone transcription (Shen et al. 1994; Arango et al. 1999). In the Leydig cells, Sf1 activates genes encoding the enzymes that make testosterone.



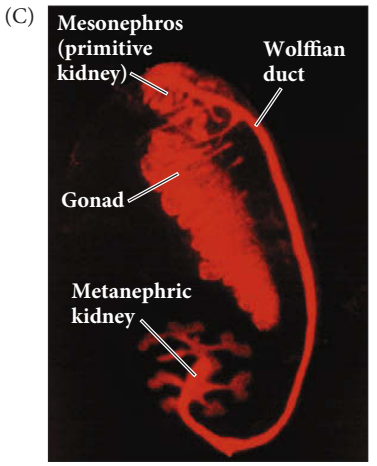




**FIGURE 6.8** Migration of mesonephric endothelial cells into $Sry^+$ gonadal rudiments. In the experiment diagrammed, urogenital ridges (containing both primitive mesonephric kidneys and bipotential gonadal rudiments) were collected from 12-day embryonic mice. Some of the mice were marked with a β-galactosidase transgene (*lacZ*) that is active in every cell. Thus, every cell of these mice turned blue when stained for β-galactosidase. The gonad and mesonephros were separated and recombined, using gonadal tissue from unlabeled mice and mesonephros from labeled mice. (A) Migration of mesonephric cells into the gonad was seen (1) when the gonadal cells were XY or when they were XX with a *Sry* transgene. No migration of mesonephric tissue into the gonad was seen (2) when the gonad contained either XX cells or XY cells in which the Y chromosome had a deletion in the *Sry* gene. The sex chromosomes of the mesonephros did not affect the migration. (B) Gonadal rudiments for XX mice could induce mesonephric cell migration if these rudiments had been incubated with Fgf9. (C) Intimate relation between the Wolffian duct and the developing gonad in a 16-day male mouse embryo. The mesonephric duct of the primitive kidney will form the efferent ducts of the testes and the Wolffian duct that leads to the ureter. The ducts and gonad have been stained for cytokeratin-8. (A,B after Capel et al. 1999, photographs courtesy of B. Capel; C from Sariola and Saarma 1999, courtesy of H. Sariola.)

**SCIENTISTS SPEAK 6.2** Dr. Blanche Capel discusses her work on the sex determination pathways of mammals.

### *The right time and the right place*

Having the right genes doesn't necessarily mean you'll get the organ you expect. Studies of mice have shown that the *Sry* gene of some strains of mice failed to produce testes when bred onto a different genetic background (Eicher and Washburn 1983; Washburn and Eicher 1989; Eicher et al. 1996). This failure can be attributed either to a delay in *Sry* expression, or to the failure of the protein to accumulate to the critical threshold level required to trigger *Sox9* expression and launch the male pathway. By the time *Sox9* gets turned on, it is too late—the gonad is already well along the path to become an ovary (Bullejos and Koopman 2005; Wilhelm et al. 2009).

The importance of timing was confirmed when Hiramatsu and collaborators (2009) were able to place the mouse *Sry* gene onto the regulatory sequences of a heat-sensitive gene, allowing them to activate *Sry* at any time in mouse development by merely raising the embryo's temperature. When they delayed *Sry* activation by as little as 6 hours, testis formation failed and ovaries started to develop (**FIGURE 6.9**). Thus, there appears to be a brief window during which the testis-forming genes can function. If this window of opportunity is missed, the ovary-forming pathway is activated.

**Hermaphrodites** are individuals in which both ovarian and testicular tissues exist; they have either ovotestes (gonads containing both ovarian and testicular tissue) or an ovary on one side and a testis on the other.[3] As seen in Figure 6.9, ovotestes can be generated when the *Sry* gene is activated later than normal. Hermaphrodites can also result in those very rare instances when a Y chromosome is translocated to an X chromosome. In those tissues where the translocated Y is on the active X chromosome, the Y chromosome will be active and the *SRY* gene will be transcribed; in cells where the Y chromosome is on the inactive X chromosome, the Y chromosome will also be inactive (Berkovitz et al. 1992; Margarit et al. 2000). Such gonadal mosaicism for expressing *SRY* can lead to the formation of a testis, an ovary, or an ovotestis, depending on the percentage of cells expressing *SRY* in the Sertoli cell precursors (see Brennan and Capel 2004; Kashimada and Koopman 2010).

[3]This anatomical phenotype is named for Hermaphroditos, a young man in Greek mythology whose beauty inflamed the ardor of the water nymph Salmacis. She wished to be united with him forever, and the gods, in their literal fashion, granted her wish. Hermaphroditism is often considered to be one of the "intersex" conditions discussed later in the chapter.

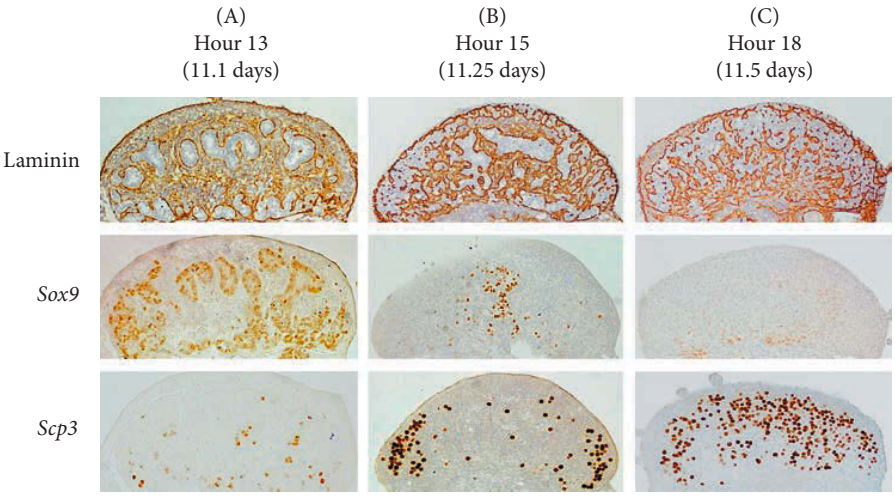



**FIGURE 6.9** Experimental delay of *Sry* gene activation by 6 hours leads to failure of testis development and the initiation of ovary development. Genital ridges were removed from XX mice carrying a heat-inducible *Sry* gene. These tissues were then heat-shocked at different times to activate *Sry* and then allowed to mature. (A) Those genital tissues experiencing *Sry* induction at 11.1 days of development (when *Sry* is normally activated) produced testes. Their laminin distribution showed Sertoli cells, *Sox9* (a marker of testis development) was active, and *Scp3*, a marker of ovary development, was absent. (B) Three hours later, the activation of *Sry* caused a central testicular area to form, with ovary-like structures forming peripherally. *Sox9* was present in the central testicular region, while *Scp3* was found in the periphery. (C) If *Sry* was activated in the genital tissues 6 hours later, the structures formed ovarian tissue, *Sox9* was absent, and *Scp3* was seen throughout the tissue. (After Hiramatsu et al. 2009.)

And just as the *Foxl2* gene is critical for the maintenance of ovarian gonadal function throughout life, the ***Dmrt1*** gene is needed for maintaining testicular structure. The deletion of *Dmrt1* in adult mice leads to the transformation of Sertoli cells into ovarian granulosa cells. Moreover, overexpression of *Dmrt1* in female mouse ovaries can reprogram the ovarian tissue into Sertoli-like cells (Lindeman et al. 2015; Zhao et al. 2015). Dmrt1 protein is probably the major male sex inducer across the entire animal kingdom, having been found in flies, cnidarians, fish, reptiles, and birds (Murphy et al. 2015; Picard et al. 2015). In mammals, SRY has taken over this function. However, these recent results show that *Dmrt1* has retained an important role in male sex determination, even in mammals.

**SCIENTISTS SPEAK 6.3** Dr. David Darkower discusses his studies showing Dmrt1 to be a major player in the male sex determination pathway.

## Secondary Sex Determination in Mammals: Hormonal Regulation of the Sexual Phenotype

Primary sex determination—the formation of either an ovary or a testis from the bipotential gonad—does not result in the complete sexual phenotype. In mammals, secondary sex determination is the development of the female and male phenotypes in response to hormones secreted by the ovaries and testes. Both female and male secondary sex determination have two major temporal phases. The first phase occurs within the embryo during organogenesis; the second occurs at puberty.

During embryonic development, hormones and paracrine signals coordinate the development of the gonads with the development of secondary sexual organs. In females, the Müllerian ducts persist and, through the actions of estrogen, differentiate to become the uterus, cervix, oviducts, and upper vagina (see Figure 6.2). The **genital tubercle** becomes differentiated into the clitoris, and the **labioscrotal folds** become the labia majora. The Wolffian ducts require testosterone to persist, and thus they atrophy in females. In females, the portion of the **urogenital sinus** that does not become the bladder and urethra becomes Skene's glands, paired organs that make secretions similar to those of the prostate.

The coordination of the male phenotype involves the secretion of two testicular factors. The first of these is anti-Müllerian hormone, a BMP-like paracrine factor made by the Sertoli cells, which causes the degeneration of the Müllerian duct. The second is the steroid hormone testosterone, an **androgen** (masculinizing substance) secreted from the fetal Leydig cells. Testosterone causes the Wolffian ducts to differentiate into sperm-carrying tubes (the epididymis and vas deferens) as well as the seminal vesicle (which emerges as an outpocketing of the vas deferens), and it causes the **genital tubercle** (the precursor of the external genitalia) to develop into the penis and the labioscrotal folds to develop into the scrotum. In males, the urogenital sinus, in addition to forming the bladder and urethra, also forms the prostate gland.

The mechanism by which testosterone (and, as we shall see, its more powerful derivative dihydrotestosterone) masculinizes the genital tubercle is thought to involve its interaction with the Wnt pathway (**FIGURE 6.10**). The Wnt pathway, which in the bipotential gonad activates the female trajectory, acts in the genital tubercle to activate male development (Mazahery et al. 2013). The Wnt antagonist Dickkopf is made in the urogenital swellings and can be downregulated by testosterone and upregulated by anti-androgens. This finding led to a model wherein the urogenital swellings of XX individuals make Dickkopf, thus preventing the activity of Wnt in the mesenchyme, blocking further growth and leading to the feminization of the genital tubercle by estrogens (Holderegger and Keefer 1986; Miyagawa et al. 2009). In females, then, the genital tubercle becomes the clitoris and the labioscrotal folds become the labia majora. In males, however, testosterone and dihydrotestosterone bind to the androgen

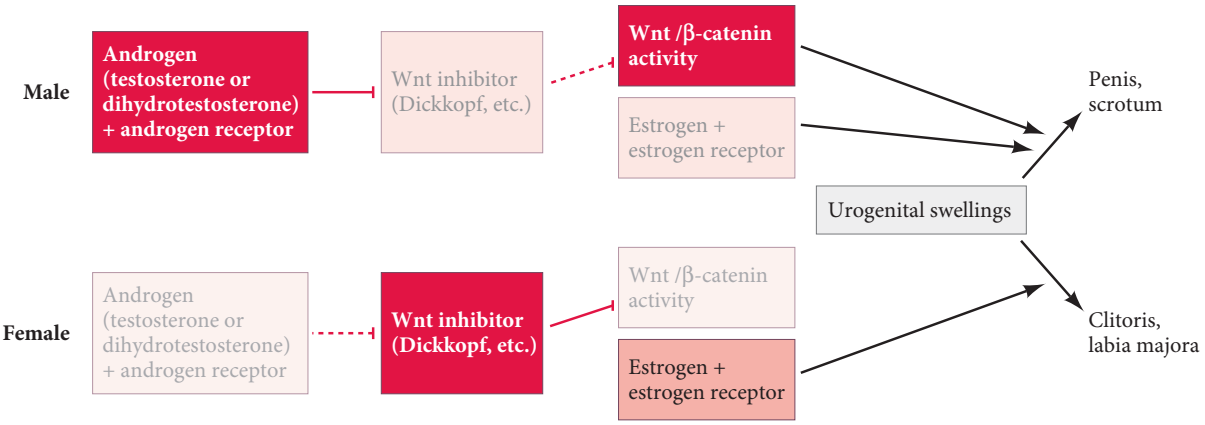



**FIGURE 6.10** Model for the formation of external genitalia. In this schema, the mesenchyme in the urogenital swellings secretes inhibitors of Wnt signaling. In the absence of Wnt signaling, estrogen modifies the genital tubercle into the clitoris and the labioscrotal folds into the labia majora surrounding the vagina. In males, however, androgens (such as testosterone and dihydrotestosterone) bind to the androgen receptor in the mesenchymal cells and prevent the synthesis of the Wnt inhibitors. Wnt signaling is permitted, and it causes the genital tubercle to become the penis and the labioscrotal folds to become the scrotum. (After Miyagawa et al. 2009.)

(testosterone) receptor in the mesenchyme and prevent the expression of Wnt inhibitors (thus permitting Wnt expression in the mesenchyme). With the influence of these Wnts, male urogenital swellings are converted into the penis and the scrotum.

WEB TOPIC 6.3 **THE ORIGINS OF GENITALIA** The cells that give rise to the penis and clitoris have only recently been identified. Their identity helps explain how male snakes get two penises and a female hyena develops a clitoris nearly as large as the male's penis.

### *The genetic analysis of secondary sex determination*

The existence of separate and independent AMH and testosterone pathways of masculinization is demonstrated by people with **androgen insensitivity syndrome**. These XY individuals, being chromosomally males, have the *SRY* gene and thus have testes that make testosterone and AMH. However, they have a mutation in the gene encoding the androgen *receptor* protein that binds testosterone and brings it into the nucleus. Therefore, these individuals cannot respond to the testosterone made by their testes (Meyer et al. 1975; Jääskeluäinen 2012). They can, however, respond to the estrogen made by their adrenal glands (which is normal for both XX and XY individuals), so they develop female external sex characteristics (**FIGURE 6.11**). Despite their distinctly female appearance, these XY individuals have testes, and even though they cannot respond to testosterone, they produce and respond to AMH. Thus, their Müllerian ducts degenerate. Persons with androgen insensitivity syndrome develop as normal-appearing but sterile women, lacking a uterus and oviducts and having internal testes in the abdomen.

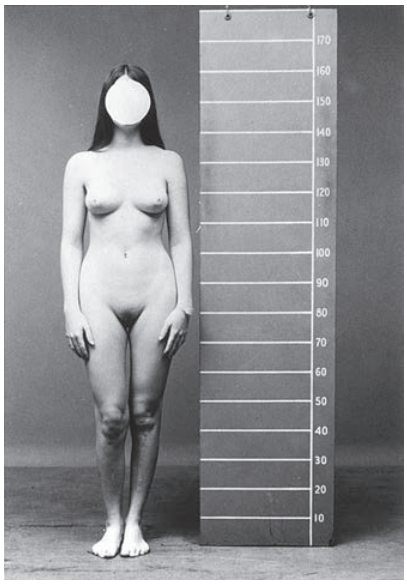


**FIGURE 6.11** Androgen insensitivity syndrome. Despite having the XY karyotype, individuals with this syndrome appear female. They cannot respond to testosterone but can respond to estrogen, so they develop female secondary sex characteristics (i.e., labia and a clitoris rather than a scrotum and a penis). Internally, they lack the Müllerian duct derivatives and have undescended testes. (Courtesy of C. B. Hammond.)

Although in most people correlation of the genetic and anatomical sexual phenotypes is high, about 0.4–1.7% of the population departs from the strictly dimorphic condition (Blackless et al. 2000; Hull 2003; Hughes et al. 2006). Phenotypes in which male and female traits are seen in the same individual are called **intersex** conditions.[4] Androgen insensitivity syndrome is one of several intersex conditions that have traditionally been labeled **pseudohermaphroditism**. In pseudohermaphrodites, there is only one type of gonad (as contrasted with true hermaphroditism, in which individuals have the gonads of both sexes), but the secondary sex characteristics differ from what would be expected from the gonadal sex. In humans, male pseudohermaphroditism (male gonadal sex with female secondary characteristics) can be caused by mutations in the androgen (testosterone) receptor or by mutations affecting testosterone synthesis (Geissler et al. 1994).

Female pseudohermaphroditism, in which the gonadal sex is female but the person is outwardly male, can be the result of overproduction of androgens in the ovary or adrenal gland. The most common cause of this latter condition is **congenital adrenal hyperplasia**, in which there is a genetic deficiency of an enzyme that metabolizes cortisol steroids in the adrenal gland. In the absence of this enzyme, testosterone-like steroids accumulate and can bind to the androgen receptor, thus masculinizing the fetus (Migeon and Wisniewski 2000; Merke et al. 2002).

**TESTOSTERONE AND DIHYDROTESTOSTERONE** Although testosterone is one of the two primary masculinizing factors, there is evidence that it is *not* the active masculinizing hormone in certain tissues. Although testosterone is responsible for promoting the formation of the male structures that develop from the Wolffian duct primordium, testosterone does not directly masculinize the urethra, prostate, penis, or scrotum. These latter functions are controlled by **5α-dihydrotestosterone**, or **DHT** (**FIGURE 6.12**). Siiteri and Wilson (1974) showed that testosterone is converted to DHT in the urogenital sinus and swellings, but not in the Wolffian duct. DHT appears to be a more potent hormone than testosterone. It is most active prenatally and in early childhood.[5]

The importance of DHT in the early development of the male gonads was demonstrated by Imperato-McGinley and her colleagues (1974) when they studied a phenotypically remarkable syndrome in several inhabitants of a small community in the Dominican Republic. Individuals with this syndrome were found to lack a functional gene for the enzyme 5α-ketosteroid reductase 2—the enzyme that converts testosterone to DHT (Andersson et al. 1991; Thigpen et al. 1992). Chromosomally XY children with this syndrome have functional testes, but the testes remain inside the abdomen and do not descend before birth. These children appear to be girls and are raised as such. Their internal anatomy, however, is male: they have Wolffian duct development and Müllerian duct degeneration, along with their functional testes. At puberty, when the testes produce high levels of testosterone (which

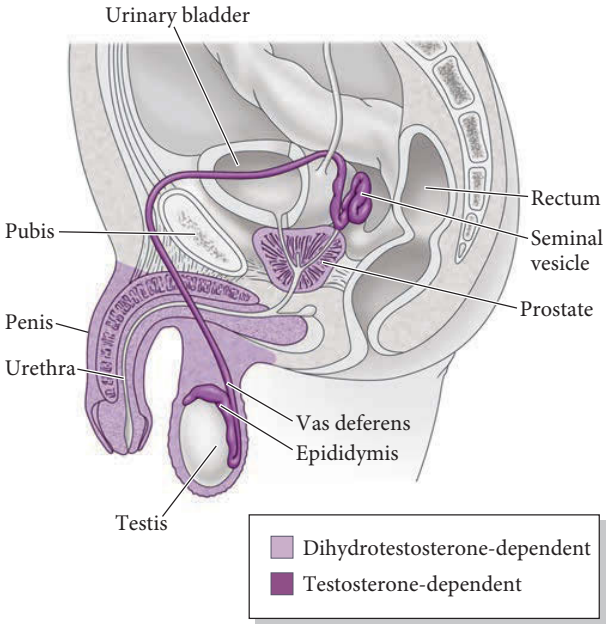



**FIGURE 6.12** Testosterone- and dihydrotestosterone-dependent regions of the human male genital system. (After Imperato-McGinley et al. 1974.)

[4]The "intersex" language used to group these conditions is being debated. Some activists, physicians, and parents wish to eliminate the term "intersex" to avoid confusion of these anatomical conditions with identity issues such as homosexuality. They prefer to call these conditions "disorders of sex development." In contrast, other activists do not want to medicalize this condition and find the "disorder" category offensive to individuals who do not feel there is anything wrong with their health. For a more detailed analysis of intersexuality, see Gilbert et al. 2005, Austin et al. 2011, and Dreger 2000.

[5]There's a reason the label on some hair-restoring drugs warns pregnant women not to handle them. Finasteride, an active ingredient in these products, blocks the metabolism of testosterone into dihydrotestosterone and thus could interfere with the gonadal development of a male fetus.

appears to compensate for the lack of DHT), their external genitalia are able to respond to the hormone and differentiate. The penis enlarges, the scrotum descends, and the person originally believed to be a girl is revealed to be a young man. Studies of this condition led to the current perception that the formation of the external genitalia is under the control of dihydrotestosterone, whereas Wolffian duct differentiation is controlled by testosterone itself.

WEB TOPIC 6.4 **DESCENT OF THE TESTES** The descent of the testes is initiated around week 10 of human pregnancy by dihydrotestosterone and another hormone from the Leydig cells, insulin-like hormone.

**ANTI-MÜLLERIAN HORMONE** Anti-Müllerian hormone, a member of the TGF-β family of growth and differentiation factors, is secreted from the fetal Sertoli cells and causes the degeneration of the Müllerian duct (Tran et al. 1977; Cate et al. 1986). AMH is thought to bind to the mesenchyme cells surrounding the Müllerian duct, causing these cells to secrete factors that induce apoptosis in the duct's epithelium and breaks down the basal lamina surrounding the duct (Trelstad et al. 1982; Roberts et al. 1999, 2002).

**ESTROGEN** The steroid hormone estrogen is needed for complete postnatal development of both the Müllerian and the Wolffian ducts, and is necessary for fertility in both males and females. In females, estrogen induces the differentiation of the Müllerian duct into the uterus, oviducts, cervix, and upper vagina. In female mice whose genes for estrogen receptors are knocked out, the germ cells die in the adult, and the granulosa cells that had enveloped them start developing into Sertoli-like cells (Couse et al. 1999). Male mice with knockouts of estrogen receptor genes produce few sperm. One of the functions of the male efferent duct cells (which bring the sperm from the seminiferous vesicles into the epididymis) is to absorb most of the water from the lumen of the rete testis. This absorption, which is regulated by estrogen, concentrates the sperm, giving them a longer life span and providing more sperm per ejaculate. If estrogen or its receptor is absent in male mice, water is not absorbed and the mouse is sterile (Hess et al. 1997). Although blood concentrations of estrogen are in general higher in females than in males, the concentration of estrogen in the rete testis is higher than in female blood.

In summary, primary sex determination in mammals is regulated by the chromosomes, which results in the production of testes in XY individuals and ovaries in XX individuals. This type of sex determination appears to be a "digital" (either/or) phenomenon. With chromosomal sex established, the gonads then produce the hormones that coordinate the different parts of the body to have a male or female phenotype. This secondary sex determination is more "analogue," where differing levels of hormones and responses to hormones can create different phenotypes. Secondary sex determination is thus usually, but not always, coordinated with the primary sex determination.

WEB TOPIC 6.5 **BRAIN SEX AND GENDER** In addition to the physical aspects of secondary sex determination, there are also behavioral attributes. The brain is an organ that differs between males and females; but does it generate a different pattern of human behaviors?

**SCIENTISTS SPEAK 6.4** Neuroscientist Dr. Daphna Joel discusses her research showing that male and female brains are remarkably similar.

## Chromosomal Sex Determination in *Drosophila*

Although both mammals and fruit flies produce XX females and XY males, the ways in which their chromosomes achieve these ends are very different. In mammals, the Y chromosome plays a pivotal role in determining the male sex. In *Drosophila*, the Y chromosome is not involved in determining sex. Rather, in flies, the Y chromosome

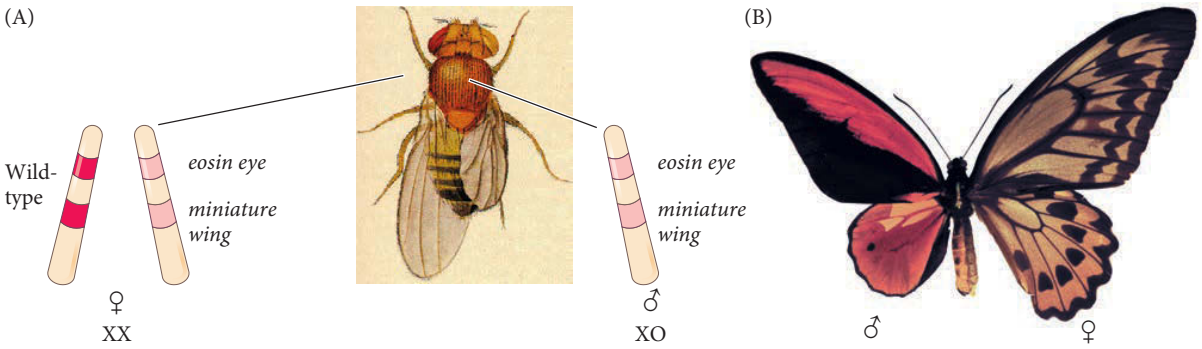



**FIGURE 6.13** Gynandromorph insects. (A) *D. melanogaster* in which the left side is female (XX) and the right side is male (XO). The male side has lost an X chromosome bearing the wild-type alleles of eye color and wing shape, thereby allowing expression of the recessive alleles *eosin eye* and *miniature wing* on the remaining X chromosome. (B) Birdwing butterfly *Ornithopera croesus*. The smaller male half is red, black, and yellow, while the female half is larger and brown. (A, drawing by Edith Wallace from Morgan and Bridges 1919; B, Montreal Insectarium, photograph by the author.)

seems to be a collection of genes that are active in forming sperm in adults, but not in sex determination.

A fruit fly's sex is determined predominantly by the number of X chromosomes in each cell. If there is only one X chromosome in a diploid cell, the fly is male. If there are two X chromosomes in a diploid cell, the fly is female. Should a fly have two X chromosomes and three sets of autosomes, it is a **mosaic**, where some of the cells are male and some of the cells are female. Thus, while XO mammals are sterile females (no Y chromosome, thus no *Sry* gene), XO *Drosophila* are sterile males (one X chromosome per diploid set).

In *Drosophila*, and in insects in general, one can observe gynandromorphs—animals in which certain regions of the body are male and other regions are female (**FIGURE 6.13**). Gynandromorph fruit flies result when an X chromosome is lost from one embryonic nucleus. The cells descended from that cell, instead of being XX (female), are XO (male). The XO cells display male characteristics, whereas the XX cells display female traits, suggesting that, in *Drosophila,* each cell makes its own sexual "decision." Indeed, in their classic discussion of gynandromorphs, Morgan and Bridges (1919) concluded, "Male and female parts and their sex-linked characters are strictly self-determining, each developing according to its own aspiration," and each sexual decision is "not interfered with by the aspirations of its neighbors, nor is it overruled by the action of the gonads." Although there are organs that are exceptions to this rule (notably the external genitalia), it remains a good general principle of *Drosophila* sexual development.

## *The Sex-lethal gene*

Although it had long been thought that a fruit fly's sex was determined by the X-to-autosome (X:A) ratio (Bridges 1925), this assessment was based largely on flies with aberrant numbers of chromosomes. Recent molecular analyses suggest that X chromosome number alone is the primary sex determinant in normal diploid insects (Erickson and Quintero 2007). The X chromosome contains genes encoding transcription factors that activate the critical gene in *Drosophila* sex determination, the X-linked locus ***Sex-lethal*** (***Sxl***). The Sex-lethal protein is a splicing factor that initiates a cascade of RNA processing events that will eventually lead to male-specific and female-specific transcription factors (**FIGURE 6.14**). These transcription factors (the Doublesex proteins) then differentially activate the genes involved to produce either the male phenotype (testes, sex combs, pigmentation) or the female phenotype (ovaries, yolk proteins, pigmentation).

**ACTIVATING *SEX-LETHAL*** The number of X chromosomes appears to have only a single function: activating (or not activating) the early expression of *Sex-lethal*.[6] *Sxl* encodes an RNA splicing factor that will regulate gonad development and will also

[6]This gene's gory name is derived from the fact that mutations of this gene can result in aberrant dosage compensation of X-linked genes (see Web Topic 6.6). As a result, there is inadequate transcription of those genes encoded on the X chromosome, and the embryo dies.

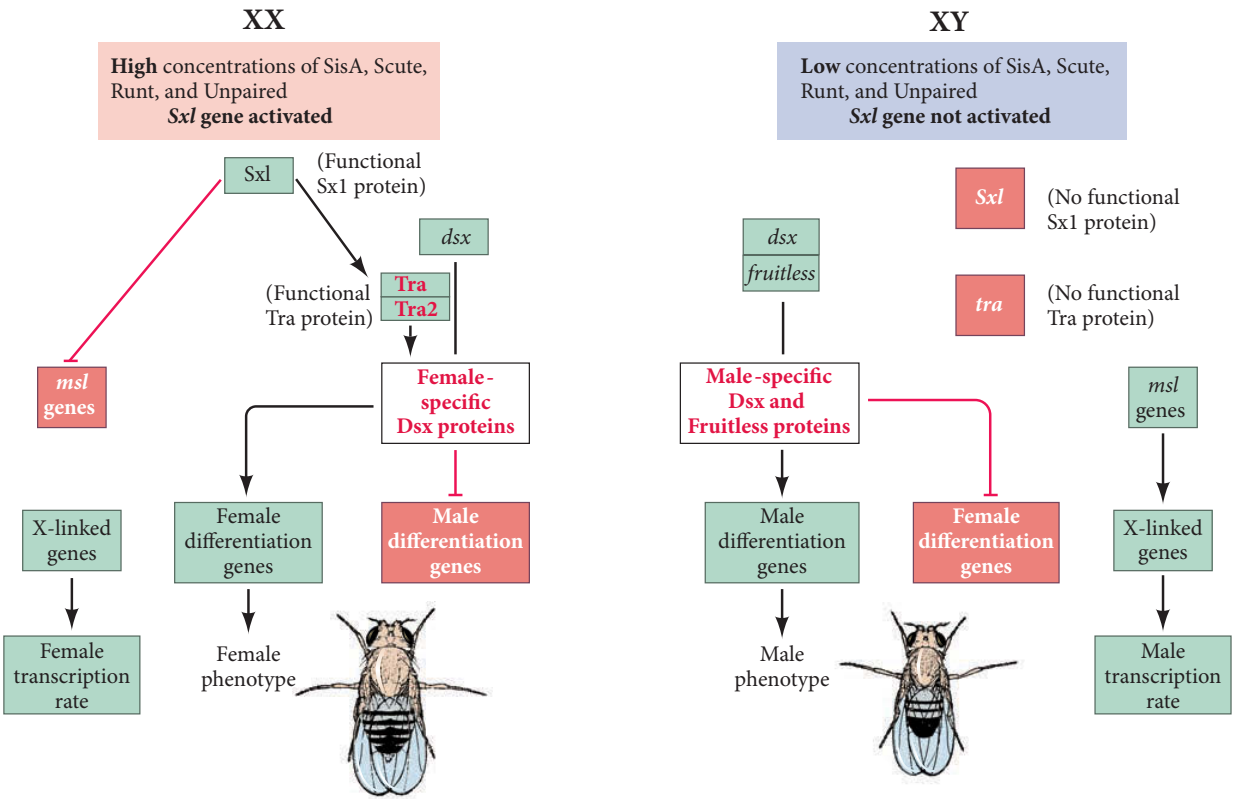



**FIGURE 6.14** Proposed regulatory cascade for *Drosophila* somatic sex determination. Transcription factors from the X chromosomes activate the *Sxl* gene in females (XX) but not in males (XY). The Sex-lethal protein performs three main functions. First, it activates its own transcription, ensuring further Sxl production. Second, it represses the translation of *msl2* mRNA, a factor that facilitates transcription from the X chromosome. This equalizes the amount of transcription from the two X chromosomes in females with that of the single X chromosome in males. Third, Sxl enables the splicing of the *transformer-1* (*tra1*) pre-mRNA into functional proteins. The Tra proteins process *doublesex* (*dsx*) pre-mRNA in a female-specific manner that provides most of the female body with its sexual fate. They also process the *fruitless* pre-mRNA in a female-specific manner, giving the fly female-specific behavior. In the absence of Sxl (and thus the Tra proteins), *dsx* and *fruitless* pre-mRNAs are processed in the male-specific manner. (The *fruitless* gene is discussed in Web Topic 6.7.) (After Baker et al. 1987.)

regulate the amount of gene expression from the X chromosome. The gene has two promoters. The early promoter is active only in XX cells; the later promoter is active in both XX and XY cells. The X chromosome appears to encode four protein factors that activate the early promoter of *Sxl*. Three of these proteins are transcription factors—SisA, Scute, and Runt—that bind to the early promoter to activate transcription. The fourth protein, Unpaired, is a secreted factor that reinforces the other three proteins through the JAK-STAT pathway (Sefton et al. 2000; Avila and Erickson 2007). If these factors accumulate so they are present in amounts above a certain threshold, the *Sxl* gene is activated through its early promoter (Erickson and Quintero 2007; Gonzáles et al. 2008; Mulvey et al. 2014). The result is the transcription of *Sxl* early in XX embryos, during the syncytial blastoderm stage.

The *Sxl* pre-RNA transcribed from the *early* promoter of XX embryos lacks exon 3, which contains a stop codon. Thus, Sxl protein that is made early is spliced in a manner such that exon 3 is absent, so early XX embryos have complete and functional Sxl protein (**FIGURE 6.15**). In XY embryos, the early promoter of *Sxl* is not active and no functional Sxl protein is present. However, later in development, as cellularization is taking place, the *late* promoter becomes active and the *Sxl* gene is transcribed in both males and females. In XX cells, Sxl protein from the early promoter can bind to its own pre-mRNA and splice it in a "female" direction. In this case, Sxl binds to and blocks the splicing complex on exon 3 (Johnson et al. 2010; Salz 2011). As a result, exon 3 is skipped and is not included in the *Sxl* mRNA. Thus, early production ensures that functional full-length (354-amino acid) Sxl protein is made if the cells are XX (Bell et al. 1991; Keyes et al. 1992). In XY cells, however, the early promoter is not active (because the X-encoded transcription factors haven't reached the threshold to activate the promoter) and there is no early Sxl protein. Therefore, the *Sxl* pre-mRNA of XY cells is spliced in a manner that *includes* exon 3 and its termination codon. Protein synthesis ends at the third exon (after amino acid 48), and the Sxl is nonfunctional.

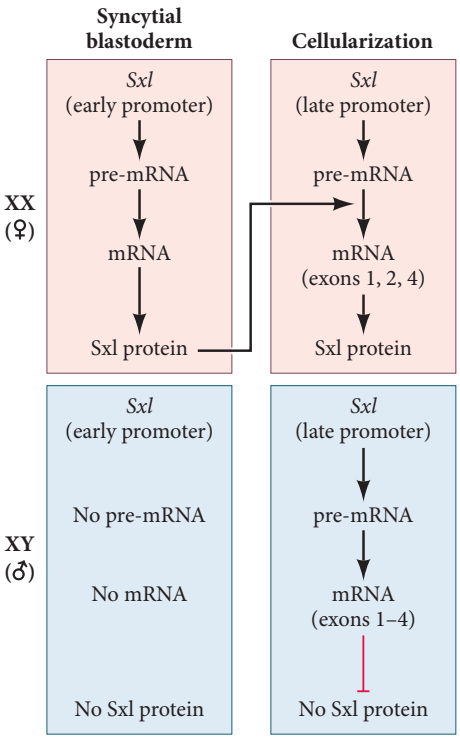



**FIGURE 6.15** Differential RNA splicing and sex-specific expression of *Sex-lethal*. In the syncytial blastoderm of XX flies, transcription factors from the two X chromosomes are sufficient to activate the early promoter of the *Sxl* gene. This "early" transcript is spliced into an mRNA lacking exon 3 and makes a functional Sxl protein. The early promoter of XY flies is not activated, and males lack functional Sxl. By the cellularizing blastoderm stage, the late promoter of *Sxl* is active in both XX and XY flies. In XX flies, Sxl already present in the embryo prevents the splicing of exon 3 into mRNA and functional Sxl protein is made. Sxl then binds to its own promoter to keep it active; it also functions to splice downstream pre-mRNAs. In XY embryos, no Sxl is present and exon 3 is spliced into the mRNA. Because of the termination codon in exon 3, males do not make functional Sxl. (After Salz 2011.)

**WEB TOPIC 6.6** **DOSAGE COMPENSATION** If the cells of female flies, nematodes, and mammals have twice the number of X chromosomes as male cells, how are the genes on the X chromosome regulated? The three groups offer three different solutions to the problem.

**TARGETS OF SEX-LETHAL** The protein made by the female-specific *Sxl* transcript contains regions that are important for binding to RNA. There appear to be three major RNA targets to which the female-specific *Sxl* transcript binds. One of these is the pre-mRNA of *Sxl* itself. Another target is the *msl2* gene that controls dosage compensation (see below). Indeed, if the *Sxl* gene is nonfunctional in a cell with two X chromosomes, the dosage compensation system will not work, and the result is cell death (hence the gene's name). The third target is the pre-mRNA of *transformer* (*tra*)—the next gene in the cascade (**FIGURE 6.16**; Nagoshi et al. 1988; Bell et al. 1991).

The pre-mRNA of *transformer* (so named because loss-of-function mutations turn females into males) is spliced into a functional mRNA by Sxl protein. The *tra* pre-mRNA is made in both male and female cells; however, in the presence of Sxl, the *tra* transcript is alternatively spliced to create a female-specific mRNA, as well as a nonspecific mRNA that is found in both females and males. Like the male *Sxl* message, the nonspecific *tra* mRNA message contains an early termination codon that renders the protein nonfunctional (Boggs et al. 1987). In *tra*, the second exon of the nonspecific mRNA contains the termination codon and is not utilized in the female-specific message (see Figures 6.14 and 6.16).

How is it that females and males make different mRNAs? The female-specific Sxl protein activates a 3′ splice site that causes *tra* pre-mRNA to be processed in a way that splices out the second exon. To do this, Sxl protein blocks the binding of splicing factor U2AF to the nonspecific splice site of the *tra* message by specifically binding to the polypyrimidine tract adjacent to it (Handa et al. 1999). This causes U2AF to bind to the lower-affinity (female-specific) 3′ splice site and generate a female-specific mRNA (Valcárcel et al. 1993). The female-specific Tra protein works in concert with the product of the *transformer-2* (*tra2*) gene to help generate the female phenotype by splicing the *doublesex* gene in a female-specific manner.

## *Doublesex: The switch gene for sex determination*

The *Drosophila* ***doublesex*** (***dsx***) gene is active in both males and females, but its primary transcript is processed in a sex-specific manner (Baker et al. 1987). This alternative RNA processing is the result of the action of the *tra* and *tra2* gene products on the *dsx* gene (see Figures 6.14 and 6.16). If the Tra2 and female-specific Tra proteins are both present, the *dsx* transcript is processed in a female-specific manner (Ryner and Baker 1991). The female splicing pattern produces a female-specific protein that activates female-specific genes (such as those of the yolk proteins) and inhibits male development. If no functional Tra is produced, a male-specific *dsx* transcript is made; this transcript encodes a transcription factor that inhibits female traits and promotes male traits. In the embryonic gonad, Dsx regulates all known aspects of sexually dimorphic gonad cell fate.

In XX flies, the female Doublesex protein ($Dsx^F$) combines with the product of the *intersex* gene (*Ix*) to make a transcription factor complex that is responsible for promoting female-specific traits. This "Doublesex complex" activates the *Wingless* (*Wg*) gene, whose Wnt-family product promotes growth of the female portions of the genital disc. It also represses the *Fgf* genes responsible for making male accessory organs, activates the genes responsible for making yolk proteins, promotes the growth of the sperm storage duct, and modifies *bricabrac* (*bab*) gene expression to give the female-specific pigmentation profile. In contrast, the male Doublesex protein ($Dsx^M$) acts directly as a transcription

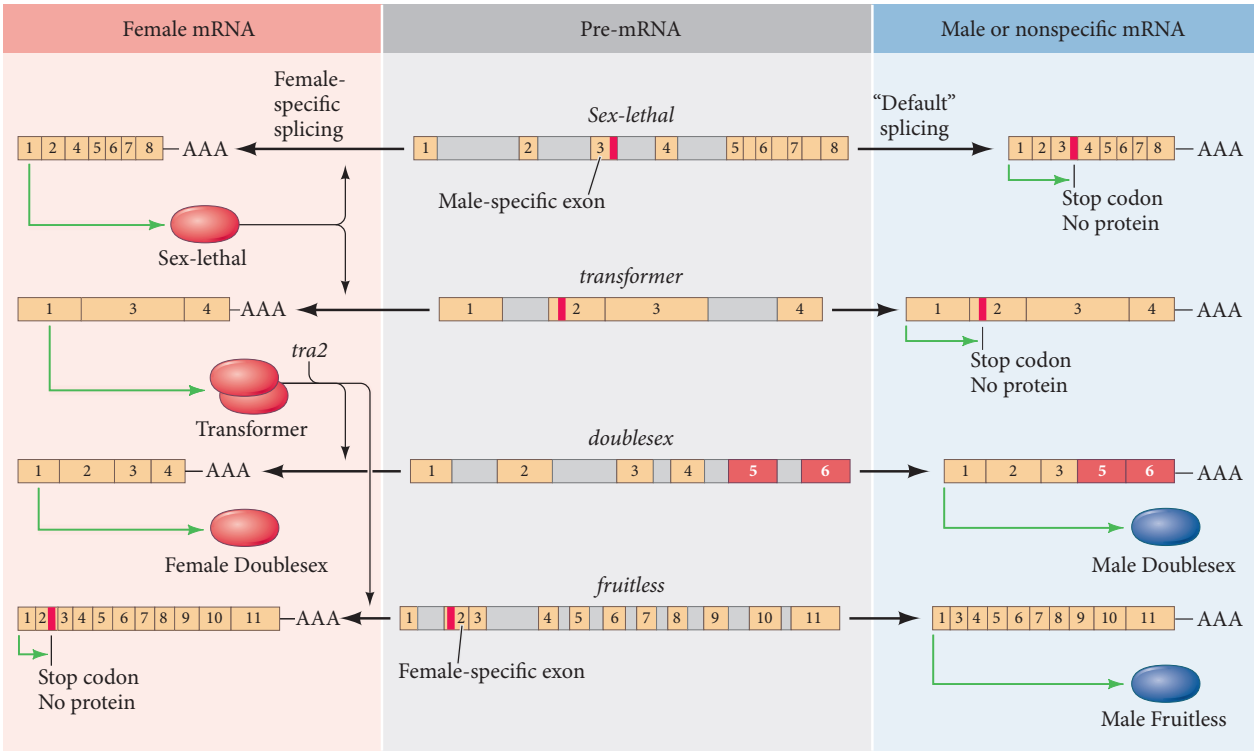



**FIGURE 6.16** Sex-specific RNA splicing in four major *Drosophila* sex-determining genes. The pre-mRNAs (shown in the center of diagram) are identical in both male and female nuclei. In each case, the female-specific transcript is shown at the left, while the default transcript (whether male or nonspecific) is shown to the right. Exons are numbered, and the positions of termination codons are marked. *Sex-lethal*, *transformer*, and *doublesex* are all part of the genetic cascade of primary sex determination. The transcription pattern of *fruitless* determines the secondary characteristic of courtship behavior. (After Baker 1989; Baker et al. 2001.)

factor and directs the expression of male-specific traits. It causes the male region of the genital disc to grow at the expense of the female disc regions. It activates the BMP homologue *Decapentaplegic* (*Dpp*), as well as stimulating *Fgf* genes to produce the male genital disc and accessory structures. $Dsx^M$ also converts certain cuticular structures into claspers and modifies the *bricabrac* gene to produce the male pigmentation pattern (Ahmad and Baker 2002; Christiansen et al. 2002).

According to this model, the result of the sex determination cascade summarized in Figure 6.14 comes down to the type of mRNA processed from the *doublesex* transcript. If there are two X chromosomes, the transcription factors activating the early promoter of *Sxl* reach a critical concentration, and *Sxl* makes a splicing factor that causes the *transformer* gene transcript to be spliced in a female-specific manner. This female-specific protein interacts with the *tra2* splicing factor, causing *dsx* pre-mRNA to be spliced in a female-specific manner. If the *dsx* transcript is not acted on in this way, it is processed in a "default" manner to make the male-specific message. Interestingly, the *doublesex* gene of flies is very similar to the *Dmrt1* gene of vertebrates, and the two types of sex determination may have some common denominators.

**WEB TOPIC 6.7** **BRAIN SEX IN *DROSOPHILA*** In addition to the "doublesex" mechanism for creating sexual phenotypes in *Drosophila*, a separate "brain sex" pathway characterized by the *fruitless* gene provides individuals with the appropriate set of courtship and aggression behaviors.

## Environmental Sex Determination

In many organisms, sex is determined by environmental factors such as temperature, location and the presence of other members of the species. Chapter 25 will discuss the importance of environmental factors on normal development; here we will just discuss one of these systems, temperature-dependent sex determination in turtles.

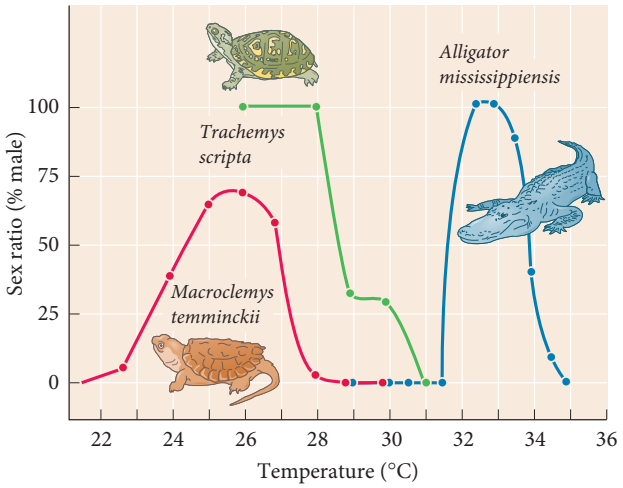



**FIGURE 6.17** Temperature-dependent sex determination in three species of reptiles: the American alligator (*Alligator mississippiensis*), red-eared slider turtle (*Trachemys scripta elegans*), and alligator snapping turtle (*Macroclemys temminckii*). (After Crain and Guillette 1998.)

While the sex of most snakes and lizards is determined by sex chromosomes at the time of fertilization, the sex of most turtles and all species of crocodylians is determined *after* fertilization, by the embryonic environment. In these reptiles, the temperature of the eggs during a certain period of development is the deciding factor in determining sex, and small changes in temperature can cause dramatic changes in the sex ratio (Bull 1980; Crews 2003). Often, eggs incubated at low temperatures produce one sex, whereas eggs incubated at higher temperatures produce the other. There is only a small range of temperatures that permits both males and females to hatch from the same brood of eggs.[7]

**FIGURE 6.17** shows the abrupt temperature-induced change in sex ratios for the red-eared slider turtle. If a brood of eggs is incubated at a temperature below 28°C, all the turtles hatching from the eggs will be male. Above 31°C, every egg gives rise to a female. At temperatures in between, the brood gives rise to individuals of both sexes. Variations on this theme also exist. The eggs of the snapping turtle *Macroclemys*, for instance, become female at either cool (22°C or lower) or hot (28°C or above) temperatures. Between these extremes, males predominate.

One of the best-studied reptiles is the European pond turtle, *Emys orbicularis*. In laboratory studies, incubating *Emys* eggs at temperatures above 30°C produces all females, whereas temperatures below 25°C produce all-male broods. The threshold temperature (at which the sex ratio is even) is 28.5°C (Pieau et al. 1994). The developmental "window" during which sex determination occurs can be discovered by incubating eggs at the male-producing temperature for a certain amount of time and then shifting them to an incubator at the female-producing temperature (and vice versa). In *Emys*, the middle third of development appears to be the most critical for sex determination, and it is believed that the turtles cannot reverse their sex after this period.

The expression of sex-determining genes (*Sox9* and *Sry* in males; *β-catenin* in females) are seen to correlate with male- or female-producing temperatures (see Mork and Chapel 2013; Bieser and Wibbels 2014). However, it is not known whether these genes are the temperature-sensitive components of sex determination. Recently, genetic studies on the sensitivity of temperature-induced sex determination have pointed to CIRBP (cold-induced RNA-binding protein) as the agent responding to temperature differences (Schroeder et al. 2016). The gene for CIRBP is expressed at the time of sex determination in snapping turtles, and different alleles give different sex ratio biases. This protein may act by repressing the splicing or translation of certain messages at certain temperatures. Another temperature-sensitive protein that may regulate sex determination is TRPV4, a $Ca^{2+}$ channel whose activity correlates with activating testes-forming genes (Yatsui et al. 2015). The mechanisms of environmentally induced sex determination have yet to be elucidated.

## Mammalian Gametogenesis

One of the most important events in sex determination is the determination of the germ cells to undergo **gametogenesis**, the formation of gametes (sperm and egg). As in the case of the genital ridges, the mammalian **primordial germ cells** (**PGCs**) are bipotential and can become either sperm or eggs; if they reside in the ovaries they become eggs, and if they reside in the testes they become sperm. All of these decisions are coordinated by factors produced by the developing gonads.

[7] The evolutionary advantages and disadvantages of temperature-dependent sex determination are discussed in Chapter 26.

First and importantly, the cells that generate the sperm or eggs do not originally form inside the gonads. Rather, they form in the posterior portion of the embryo and migrate into the gonads (Anderson et al. 2000; Molyneaux et al. 2001; Tanaka et al. 2005). This pattern is common throughout the animal kingdom: the germ cells are "set aside" from the rest of the embryo and the cells' transcription and translation are shut down while they migrate from peripheral sites into the embryo and to the gonad. It is as if the germ cells were a separate entity, reserved for the next generation, and repressing gene expression makes them insensitive to the intercellular commerce going on all around them (Richardson and Lehmann 2010; Tarbashevich and Raz 2010).

Although the mechanisms used to specify the germ cells vary enormously across the animal kingdom, the proteins expressed by germ cells to suppress gene expression are remarkably conserved. These proteins, which include the Vasa, Nanos, Tudor, and Piwi family proteins, can be seen in the germ cells of cnidarians, flies, and mammals (Ewen-Campen et al. 2010; Leclére et al. 2012). **Vasa** proteins are required for germ cells in nearly all animals studied. They are involved in binding RNA and most likely activate germ-cell-specific messages. In chickens, experimentally induced Vasa can direct embryonic stem cells toward a germ cell fate (Lavial et al. 2009). **Nanos** proteins bind to their partner, Pumilio, to form a very potent repressive dimer. Nanos can block RNA translation, and Pumilio binds to the 3′ UTRs of specific mRNAs. In *Drosophila*, Nanos and Pumilio repress the translation of numerous mRNAs, and in so doing they (1) prevent the cell from becoming part of any germ layer; (2) prevent the cell cycle from continuing; and (3) prevent apoptosis (Kobayashi et al. 1996; Asaoka-Taguchi et al. 1999; Hayashi et al. 2004). **Tudor** proteins were discovered in *Drosophila*, in which females carrying these genes are sterile[8] and do not form pole cells (Boswell and Mahowald 1985). It appears that Tudor proteins interact with those **Piwi** proteins that are involved in transcriptionally silencing portions of the genome, especially active transposons.

**WEB TOPIC 6.8 THEODOR BOVERI AND THE FORMATION OF THE GERM LINE** In the early 1900s, Boveri's studies on the development of roundworms demonstrated that the cytoplasm of the cell destined to be the germ cell precursor was different from the cytoplasm of other cells.

The newly formed PGCs first enter into the hindgut (**FIGURE 6.18A**) and eventually migrate forward and into the bipotential gonads, multiplying as they migrate. From the time of their specification until they enter the genital ridges, the PGCs are surrounded by cells secreting stem cell factor (SCF). SCF is necessary for PGC motility and survival. Moreover, the cluster of SCF-secreting cells appears to migrate with the PGCs, forming a "traveling niche" of cells that support the persistence, the division, and movement of the PGCs (Gu et al. 2009).

The PGCs that migrate to the gonads do not make their own decision to become either sperm or eggs. That decision is made by the gonad in which they reside; it is signals from the gonad that create the profound differences between spermatogenesis and oogenesis (**TABLE 6.1**). One of the most fundamental differences involves the *timing* of meiosis. In females, meiosis begins in the *embryonic* gonads. In males, meiosis is not initiated until puberty. The "gatekeeper" for meiosis appears to be the Stra8 transcription factor, which promotes a new round of DNA synthesis and meiotic initiation in the germ cells. In the developing ovaries, Stra8 is *upregulated* by two factors—Wnt4 and retinoic acid—coming from the adjacent kidney (Baltus et al. 2006; Bowles et al. 2006; Naillat et al. 2010; Chassot et al. 2011). In the developing testes, however, Stra8 is *downregulated* by Fgf9, and the retinoic acid produced by the mesonephros is degraded by the testes' secretion of the RA-degrading enzyme Cyp26b1 (**FIGURE 6.19**; Bowles et al. 2006; Koubova et al. 2006). During male puberty, however, retinoic acid is synthesized

[8] Tudor and Vasa are both named after European royal houses that came to an end with female monarchs (Elizabeth of England and Christina of Sweden) who had no heirs.

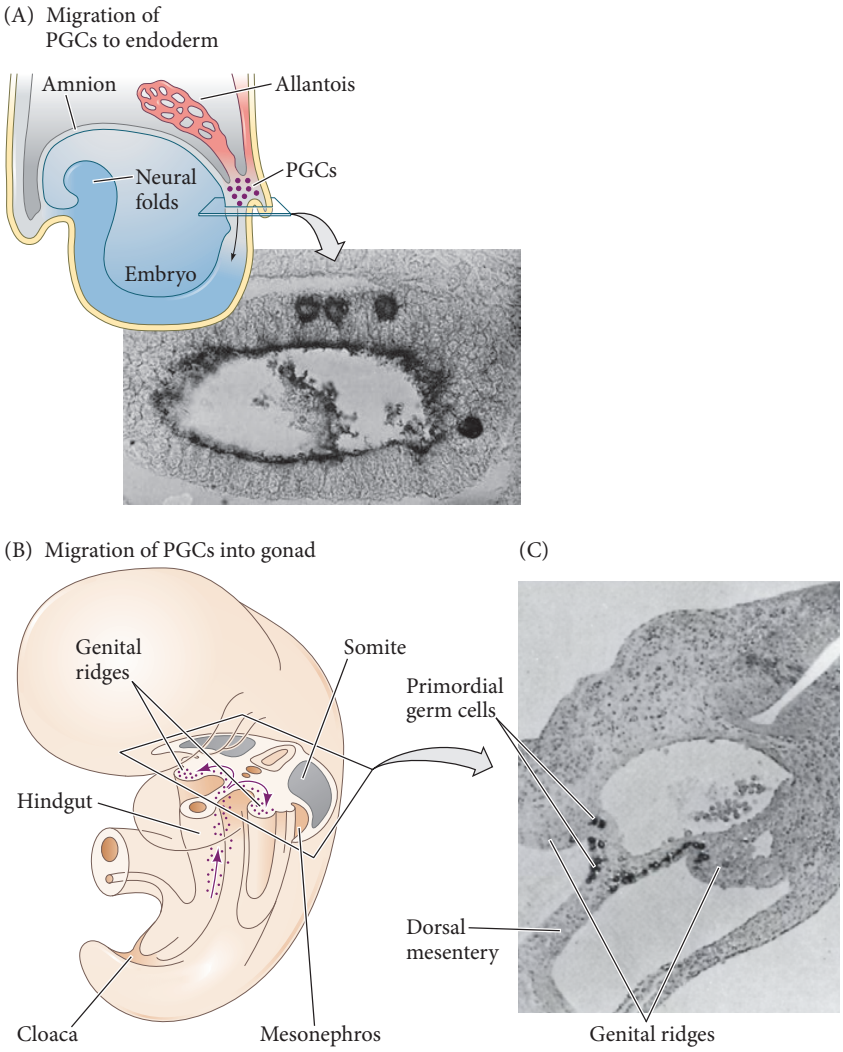



**FIGURE 6.18** Primordial germ cell migration in the mouse. (A) On embryonic day 8, PGCs established in the posterior epiblast migrate into the definitive endoderm of the embryo. The photo shows four large PGCs (stained for alkaline phosphatase) in the hindgut of a mouse embryo. (B) The PGCs migrate through the gut and, dorsally, into the genital ridges. (C) Alkaline phosphatase-staining cells are seen entering the genital ridges around embryonic day 11. (A from Heath 1978; C from Mintz 1957, courtesy of the authors.)

**TABLE 6.1 Sexual dimorphism in mammalian meioses**

| Female oogenesis | Male spermatogenesis |
|---|---|
| Meiosis initiated once in a finite population of cells | Meiosis initiated continuously in a mitotically dividing stem cell population |
| One gamete produced per meiosis | Four gametes produced per meiosis |
| Completion of meiosis delayed for months or years | Meiosis completed in days or weeks |
| Meiosis arrested at first meiotic prophase and reinitiated in a smaller population of cells | Meiosis and differentiation proceed continuously without cell cycle arrest |
| Differentiation of gamete occurs while diploid, in first meiotic prophase | Differentiation of gamete occurs while haploid, after meiosis ends |
| All chromosomes exhibit equivalent transcription and recombination during meiotic prophase | Sex chromosomes excluded from recombination and transcription during first meiotic prophase |

*Source:* After Handel and Eppig 1998.

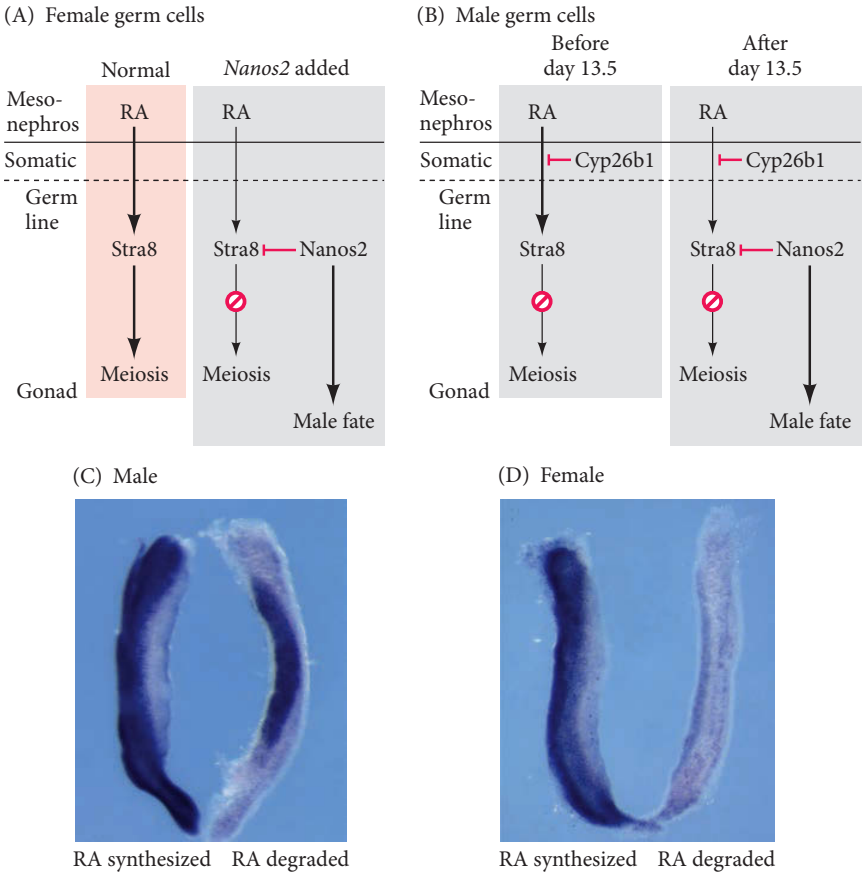



**FIGURE 6.19** Retinoic acid (RA) determines the timing of meiosis and sexual differentiation of mammalian germ cells. (A) In female mouse embryos, RA secreted from the mesonephros reaches the gonad and triggers meiotic initiation via the induction of Stra8 transcription factor in female germ cells (beige). However, if activated *Nanos2* genes are added to female germ cells, they suppress Stra8 expression, leading the germ cells into a male pathway (gray). (B) In embryonic testes, Cyp26b1 blocks RA signaling, thereby preventing male germ cells from initiating meiosis until embryonic day 13.5 (left panel). After embryonic day 13.5, when Cyp26b1 expression is decreased, Nanos2 is expressed and prevents meiotic initiation by blocking Stra8 expression. This induces male-type differentiation in the germ cells (right panel). (C,D) Day 12 mouse embryos stained for mRNAs encoding the RA-synthesizing enzyme Aldh1a2 (left gonad) and the RA-degrading enzyme Cyp26b1 (right gonad). The RA-synthesizing enzyme is seen in the mesonephros of both the male (C) and female (D); the RA-degrading enzyme is seen only in the male gonad. (A,B from Saga 2008; C,D from Bowles et al. 2006, courtesy of P. Koopman.)

in the Sertoli cells and induces Stra8 in sperm stem cells. Once Stra8 is present, the sperm stem cells become committed to meiosis (Anderson et al. 2008; Mark et al. 2008). Thus, the timing of retinoic acid synthesis appears to control Stra8, and Stra8 commits germ cells to meiosis. Fgf9, which downregulates Stra8, also appears to be critical in keeping the male germ cells in a stem cell-like condition (Bowles et al. 2010).

The structure of the mammalian gonad plays a critical role as well. The Sertoli cells, Leydig cells, and blood vessels of the seminiferous tubules constitute a stem cell niche (Hara et al. 2014; Manku and Culty 2015.) The primordial germ cells that enter the developing testis will remain in a stem cell-like condition that enables them to mitotically produce sperm precursors. The follicle cells of the ovary, however, do not constitute a stem cell niche. Rather, each primordial germ cell will be surrounded by the follicle cells, and usually only one egg will mature from each follicle.

## *Meiosis: The intertwining of life cycles*

**Meiosis** is perhaps the most revolutionary invention of eukaryotes, for it is the mechanism for transmission of genes from one generation to the next and for the recombination of sperm- and egg-derived genes into new combinations of alleles. Van Beneden's 1883 observations that the divisions of germ cells caused the resulting gametes to contain half the diploid number of chromosomes "demonstrated that the chromosomes of the offspring are derived in equal numbers from the nuclei of the two conjugating germ-cells and hence equally from the two parents" (Wilson 1924). Meiosis is a critical starting and ending point in the cycle of life. The body senesces and dies, but the gametes formed by meiosis survive the death of their parents and form the next generation.

Sexual reproduction, evolutionary variation, and the transmission of traits from one generation to the next all come down to meiosis. So to understand what germ cells do, we must first understand meiosis.

Meiosis is the means by which the gametes halve the number of their chromosomes. In the haploid condition, each chromosome is represented by only one copy, whereas diploid cells have two copies of each chromosome. Meiotic division differ from mitotic division in that (1) meiotic cells undergo two cell divisions without an intervening period of DNA replication, and (2) homologous chromosomes pair together and recombine genetic material.

After the germ cell's final mitotic division, a period of DNA synthesis occurs, so that the cell initiating meiosis doubles the amount of DNA in its nucleus. In this state, each chromosome consists of two sister **chromatids** attached at a common kinetochore.[9] (In other words, the diploid nucleus contains four copies of each chromosome.) In the first of the two meiotic divisions (meiosis I), homologous chromosomes (for example, the two copies of chromosome 3 in the diploid cell) come together and are then separated into different cells. Hence the first meiotic division *splits two homologous chromosomes between two daughter cells* such that each daughter cell has only one copy of each chromosome. But each of the chromosomes has already replicated (i.e., each has two chromatids), so the second division (meiosis II) *separates the two sister chromatids from each other.* The net result of meiosis is four cells, each of which has a single (haploid) copy of each chromosome.

The first meiotic division begins with a long prophase, which is subdivided into four stages (**FIGURE 6.20**). During the **leptotene** (Greek, "thin thread") stage, the chromatin of the chromatids is stretched out very thinly, and it is not possible to identify individual chromosomes. DNA replication has already occurred, however, and each chromosome consists of two parallel chromatids. At the **zygotene** (Greek, "yoked threads") stage, homologous chromosomes pair side by side. This pairing, called **synapsis**, is characteristic of meiosis; such pairing does not occur during mitotic divisions. Although the mechanism whereby each chromosome recognizes its homologue is not known (see Barzel and Kupiec 2008; Takeo et al. 2011), synapsis seems to require the presence of

[9] The terms *centromere* and *kinetochore* are often used interchangeably, but in fact the kinetochore is the complex protein structure that assembles on a sequence of DNA known as the centromere.

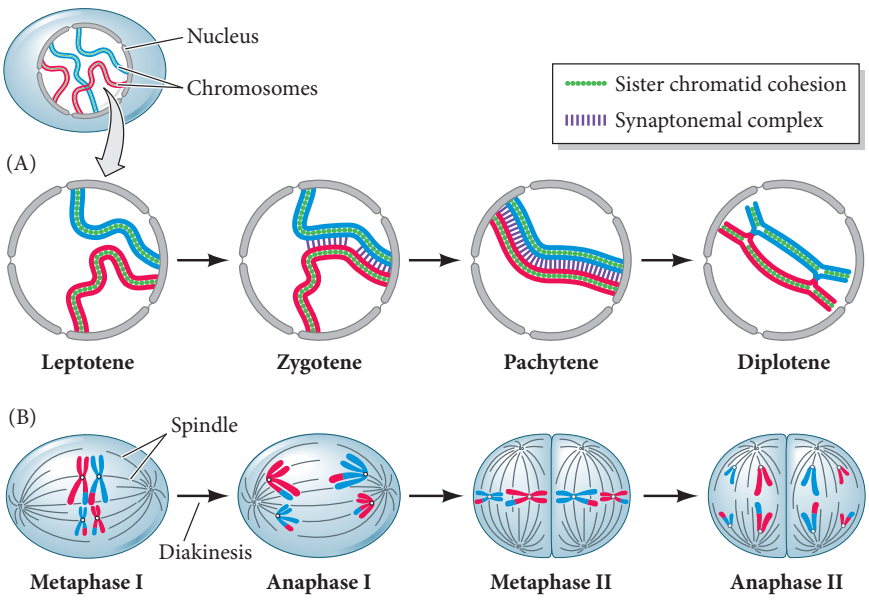



**FIGURE 6.20** Meiosis, emphasizing the synaptonemal complex. Before meiosis, unpaired homologous chromosomes are distributed randomly within the nucleus. (A) At leptotene, telomeres have attached along the nuclear envelope. The chromosomes "search" for homologous chromosomes, and synapsis, the association of homologous chromosomes, begins at zygotene, where the first evidence of the synaptonemal complex (SC) can be seen. During pachytene, homologue alignment is seen along the entire length of the chromosomes and produces a bivalent structure. Paired homologs can recombine with each (cross over) other during zygotene and pachytene. The synaptonemal complex dissolves at diplotene, when recombination is completed. (B) In diakinesis, chromosomes condense further and then form a metaphase plate. Segregation of the homologous chromosomes occurs at anaphase I. Only one pair of sister chromatids is shown here in meiosis II, where sister chromatids align at metaphase II and then in anaphase II segregate to opposite poles. (After Tsai and McKee 2011.)

the nuclear envelope and the formation of a proteinaceous ribbon called the **synaptonemal complex**. In many species, the nuclear envelope probably serves as an attachment site for the prophase chromosomes to bind and thereby reduces the complexity of the search for the other homologous chromosome (Comings 1968; Scherthan 2007; Tsai and McKee 2011). The synaptonemal complex is a ladderlike structure with a central element and two lateral bars (von Wettstein 1984; Yang and Wang 2009). The homologous chromosomes become associated with the two lateral bars, and the chromosomes are thus joined together. The configuration formed by the four chromatids and the synaptonemal complex is referred to as a **tetrad** or a **bivalent**.

During the next stage of meiotic prophase, **pachytene** (Greek, "thick thread"), the chromatids thicken and shorten. Individual chromatids can now be distinguished under the light microscope, and crossing-over may occur. **Crossing-over** represents an exchange of genetic material whereby genes from one chromatid are exchanged with homologous genes from another. Crossing-over may continue into the next stage, **diplotene** (Greek, "double threads"). During diplotene, the synaptonemal complex breaks down and the two homologous chromosomes start to separate. Usually, however, they remain attached at various points called **chiasmata**, which are thought to represent regions where crossing-over is occurring. The diplotene stage is characterized by a high level of gene transcription.

Meiotic metaphase begins with **diakinesis** (Greek, "moving apart") of the chromosomes (Figure 6.20B). The nuclear envelope breaks down and the chromosomes migrate to form a metaphase plate. Anaphase of meiosis I does not commence until the chromosomes are properly aligned on the mitotic spindle fibers. This alignment is accomplished by proteins that prevent cyclin B from being degraded until after all the chromosomes are securely fastened to microtubules.

During anaphase I, the homologous chromosomes separate from each other in an independent fashion. This stage leads to telophase I, during which two daughter cells are formed, each cell containing one partner of each homologous chromosome pair. After a brief **interkinesis**, the second meiotic division takes place. During meiosis II, the kinetochore of each chromosome divides during anaphase so that each of the new cells gets one of the two chromatids, the final result being the creation of four haploid cells. Note that meiosis has also reassorted the chromosomes into new groupings. First, each of the four haploid cells has a different assortment of chromosomes. Humans have 23 different chromosome pairs; thus $2^{23}$ (nearly 10 million) different haploid cells can be formed from the genome of a single person. In addition, the crossing-over that occurs during the pachytene and diplotene stages of first meiotic metaphase further increases genetic diversity and makes the number of potential different gametes incalculably large.

This organization and movement of meiotic chromosomes is choreographed by a ring of **cohesin proteins** that encircles the sister chromatids. Cohesin rings resist the pulling forces of the spindle microtubules, thereby keeping the sister chromatids attached during meiosis I (Haering et al. 2008; Brar et al. 2009). The cohesins also recruit other sets of proteins that help promote pairing between homologous chromosomes and allow recombination to occur (Pelttari et al. 2001; Villeneuve and Hillers 2001; Sakuno and Watanabe 2009). At the second meotic division, the cohesin ring is cleaved and the kinetochores can separate from each other (Schöckel et al. 2011).

**WEB TOPIC 6.9** **MODIFICATIONS OF MEIOSIS** In many organisms, females can reproduce themselves without males by modifying meiosis. They can produce diploid eggs and activate them by some means other than sperm entry.

### *Gametogenesis in mammals: Spermatogenesis*

**Spermatogenesis**—the developmental pathway from germ cell to mature sperm—begins at puberty and occurs in the recesses between the Sertoli cells (**FIGURE 6.21**). Spermatogenesis is divided into three major phases (Matson et al. 2010):

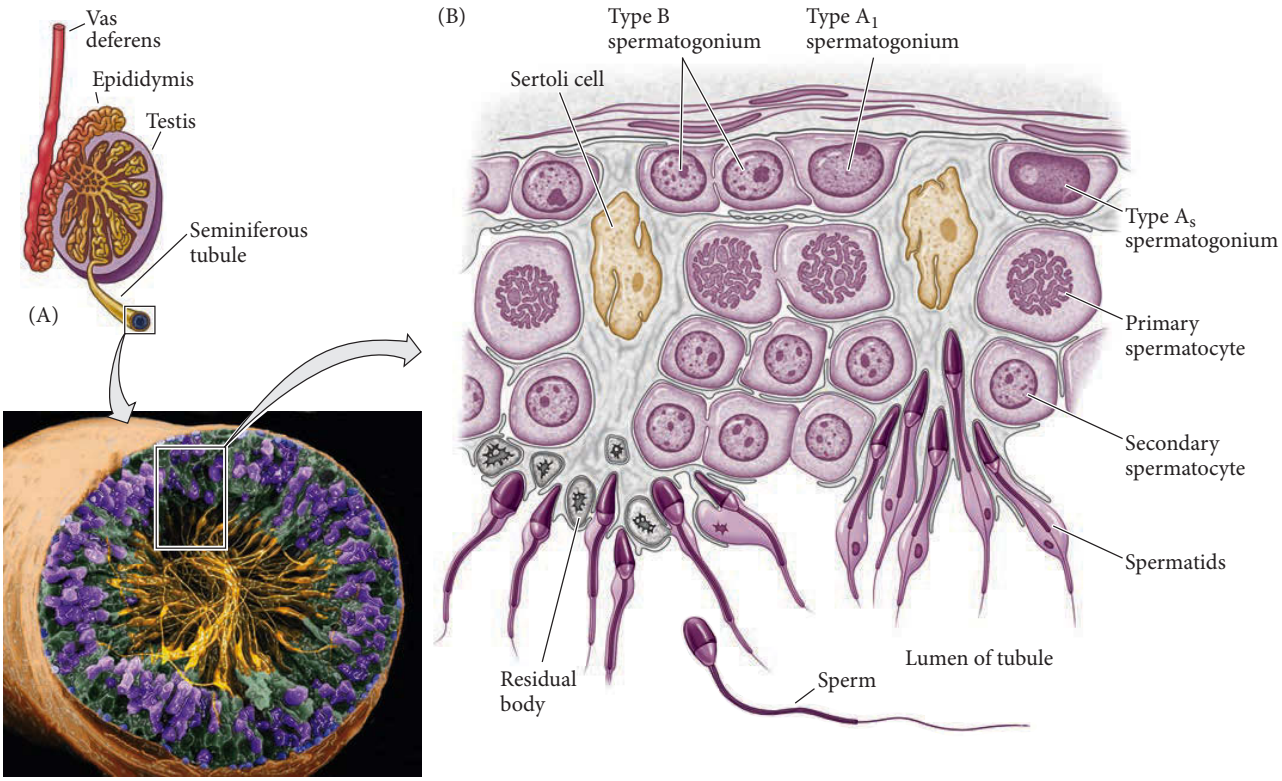



**FIGURE 6.21** Sperm maturation. (A) Cross section of the seminiferous tubule. Spermatogonia are blue, spermatocytes are lavender, and the mature sperm appear yellow. (B) Simplified diagram of a portion of the seminiferous tubule, illustrating relationships between spermatogonia, spermatocytes, and sperm. As these germ cells mature, they progress toward the lumen of the seminiferous tubule. (See also Figure 7.1.) (A photograph courtesy of R. Wagner; B based on Dym 1977.)

1. A proliferative phase where sperm stem cells (**spermatogonia**) increase by mitosis.
2. A meiotic phase, involving the two divisions that create the haploid state.
3. A postmeiotic "shaping" phase called **spermiogenesis**, during which the round cells (spermatids) eject most of their cytoplasm and become the streamlined sperm.

The proliferative phase begins when the mammalian PGCs arrive at the genital ridge of a male embryo. Here they are called **gonocytes** and become incorporated into the sex cords that will become the seminiferous tubules (Culty 2009). The gonocytes become undifferentiated spermatogonia residing near the basal end of the tubular cells (Yoshida et al. 2007, 2016). These are true stem cells in that they can reestablish spermatogenesis when transferred into mice whose sperm production was eliminated by toxic chemicals. Spermatogonia appear to take up residence in stem cell niches at the junction of the Sertoli cells (the epithelium of the seminiferous tubules), the interstitial (testosterone-producing) Leydig cells, and the testicular blood vessels. Adhesion molecules join the spermatogonia directly to the Sertoli cells, which will nourish the developing sperm (Newton et al. 1993; Pratt et al. 1993; Kanatsu-Shinohara et al. 2008). The mitotic proliferation of these stem cells amplifies this small population into a population of differentiating spermatogonia (**type A spermatogonia**) that can generate more than 1000 sperm per second in adult human males (Matson et al. 2010).

As the spermatogonia divide, they remain attached to each other by cytoplasmic bridges. But these bridges are fragile, and when one cell splits from the others, it can become an undifferentiated spematogonia again (Hara et al. 2014.) The meiotic phase of spermatogenesis during puberty is regulated by several factors. Glial cell line-derived neurotrophic factor, (GDNF, a paracrine factor) is made by the Sertoli cells and by the myoid cells that surround the tubules and give them strength and elasticity. GDNF helps keep the spermatogonia dividing as stem cells (Chen et al. 2016a). As mentioned earlier, at puberty retinoic acid levels activate the Stra8 transcription factor, and levels of

the BMP8b paracrine factor reach a critical concentration. BMP8b is thought to instruct the spermatogonia to produce receptors that enable them to respond to proteins such as stem cell factor (SCF). Indeed, mice lacking BMP8b do not initiate spermatogenesis at puberty (Zhao et al. 1996; Carlomagno et al. 2010). The transition between mitotically dividing spermatogonia and the spermatocytes that initiate meiosis appears to be mediated by the opposing influences of GDNF and SCF, both of which are secreted by the Sertoli cells. SCF promotes the transition to spermatogenesis, while GDNF promotes the division of spermatogonial stem cells (Rossi and Dolci 2013).

**THE MEIOTIC PHASE: HAPLOID SPERMATIDS** Spermatogonia with high levels of Stra8 and responding to SCF divide to become **type B spermatogonia**. (**FIGURE 6.22**; de Rooij and Russell 2000; Nakagawa 2010; Griswold et al. 2012). Type B spermatogonia are the precursors of the spermatocytes and are the last cells of the line that undergo mitosis. They divide once to generate the **primary spermatocytes**—the cells that enter meiosis. Each primary spermatocyte undergoes the first meiotic division to yield a pair of **secondary spermatocytes**, which complete the second division of meiosis. The

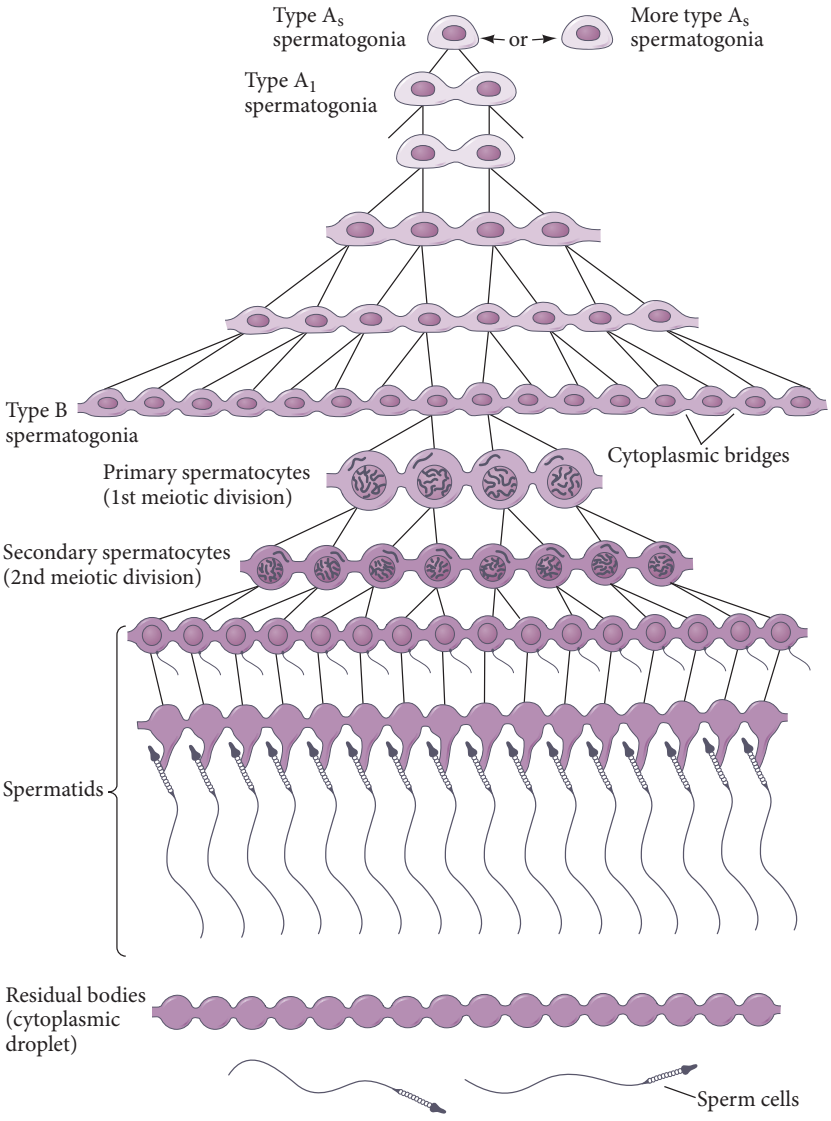



**FIGURE 6.22** Formation of syncytial clones of human male germ cells. (After Bloom and Fawcett 1975.)

haploid cells thus formed are called **spermatids**, and they are still connected to one another through their cytoplasmic bridges. The spermatids that are connected in this manner have haploid nuclei but are functionally diploid, since a gene product made in one cell can readily diffuse into the cytoplasm of its neighbors (Braun et al. 1989).

During the divisions from undifferentiated spermatogonia to spermatids, the cells move farther and farther away from the basal lamina of the seminiferous tubule and closer to its lumen (see Figure 6.21; Siu and Cheng 2004). As the spermatids move toward border of the lumen, they lose their cytoplasmic connections and differentiate into spermatozoa. In humans, the progression from spermatogonial stem cell to mature spermatozoa takes 65 days (Dym 1994).

**SPERMIOGENESIS: THE DIFFERENTIATION OF THE SPERM** The mammalian haploid spermatid is a round, unflagellated cell that looks nothing like the mature vertebrate sperm. The next step in sperm maturation, then, is **spermiogenesis** (sometimes called spermateliosis), the differentiation of the sperm cell. For fertilization to occur, the sperm has to meet and bind with an egg, and spermiogenesis prepares the sperm for these functions of motility and interaction. The process of mammalian sperm differentiation is shown in Figure 7.1. The first step is the construction of the acrosomal vesicle from the Golgi apparatus, a process about which we know very little (see Berruti and Paiardi 2011). The acrosome forms a cap that covers the sperm nucleus. As the acrosomal cap is formed, the nucleus rotates so that the cap faces the basal lamina of the seminiferous tubule. This rotation is necessary because the flagellum, which is beginning to form from the centriole on the other side of the nucleus, will extend into the lumen of the seminiferous tubule. During the last stage of spermiogenesis, the nucleus flattens and condenses, the remaining cytoplasm (the residual body, or cytoplasmic droplet; see Figure 6.22) is jettisoned, and the mitochondria form a ring around the base of the flagellum.

During spermiogenesis, the histones of the spermatogonia are often replaced by sperm-specific histone variants, and widespread nucleosome dissociation takes place. This remodeling of nucleosomes might also be the point at which the PGC pattern of methylation is removed and the male genome-specific pattern of methylation is established on the sperm DNA (see Wilkins 2005). As spermiogenesis ends, the histones of the haploid nucleus are eventually replaced by protamines.[10] This replacement results in the complete shutdown of transcription in the nucleus and facilitates the nucleus assuming an almost crystalline structure (Govin et al. 2004). The resulting sperm then enter the lumen of the seminiferous tubule.

Unexpectedly, the sperm continue to develop after they leave the testes. When being transported from the testes, sperm reside in the epididymis. During this residence, the epididymal cells release exosomes that fuse with the sperm. These exosomes have been shown to contain small ncRNAs and other factors that can activate and repress certain genes, and the sperm will bring these agents into the egg (Sharma et al. 2016; Chen et al. 2016). And the sperm still isn't fully mature, even when it exits the urethra. The final differentiation of the sperm, as we will see in Chapter 7, occurs in the reproductive tract of the female. Here, secretions from the oviducts will change the sperm cell membrane so that it can fuse with the membrane of the egg cell. Thus, the full differentiation of the sperm take place in two different organisms.

In the mouse, development from stem cell to spermatozoon takes 34.5 days: the spermatogonial stages last 8 days, meiosis lasts 13 days, and spermiogenesis takes another 13.5 days. Human sperm development takes nearly twice as long. Each day,

[10] *Protamines* are relatively small proteins that are over 60% arginine. Transcription of the genes for protamines is seen in the early haploid spermatids, although translation is delayed for several days (Peschon et al. 1987). The replacement, however, is not complete, and "activating" nucleosomes, having trimethylated H3K4, cluster around developmentally significant loci, including Hox gene promoters, certain microRNAs, and imprinted loci that are paternally expressed (Hammoud et al. 2009).

some 100 million sperm are made in each human testicle, and each ejaculation releases 200 million sperm. Unused sperm are either resorbed or passed out of the body in urine. During his lifetime, a human male can produce $10^{12}$ to $10^{13}$ sperm (Reijo et al. 1995).

### *Gametogenesis in mammals: Oogenesis*

Mammalian oogenesis (egg production) differs greatly from spermatogenesis. The eggs mature through an intricate coordination of hormones, paracrine factors, and tissue anatomy. Mammalian egg maturation can be seen as having four stages. First, there is the stage of proliferation. In the human embryo, the thousand or so PGCs reaching the developing ovary divide rapidly from the second to the seventh month of gestation. They generate roughly 7 million **oogonia** (**FIGURE 6.23**). While most of these oogonia die soon afterward, the surviving population, under the influence of retinoic acid, enter then next step and initiate the first meiotic division. They become **primary oocytes.** This first meiotic division does not proceed very far, and the primary oocytes and remain in the diplotene stage of first meiotic prophase (Pinkerton et al. 1961). This prolonged diplotene stage is sometimes referred to as the **dictyate resting stage**. This may last from 12 to 40 years. With the onset of puberty, groups of oocytes periodically resume meiosis. At that time, **luteneizing hormone** (**LH**) from the pituitary gland releases this block and permits these oocytes to resume meiotic division (Lomniczi et al. 2013). They complete first meiotic division and proceed to second meiotic metaphase. This LH surge causes the oocyte to mature. The oocyte begins to synthesize the proteins that make it competent to fuse with the sperm cell and that enable the first cell divisions of the early embryo. This maturation involves the cross-talk of paracrine factors between the oocyte and its follicular cells, both of which are maturing during this phase. The follicle cells activate the translation of stored oocyte mRNA encoding proteins such as the sperm-binding proteins that will be used for fertilization and the cyclins that control embryonic cell division (Chen et al. 2013; Cakmak et al. 2016). After the secondary oocyte is released from the ovary, meiosis will resume only if fertilization occurs. At fertilization, calcium ions are released in the egg, and these calcium ions release the inhibitory block and allow the haploid nucleus to form.

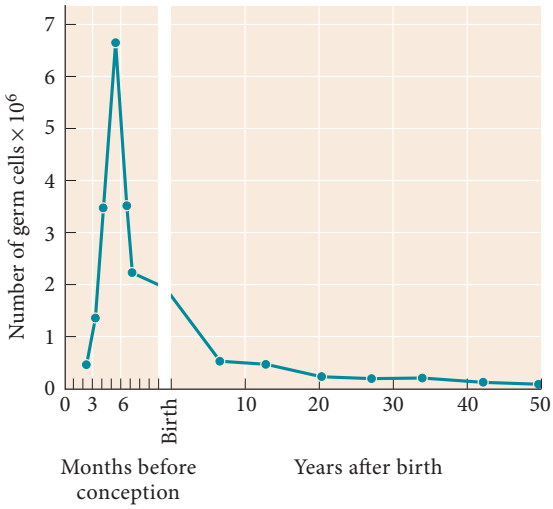



**FIGURE 6.23** The number of germ cells in the human ovary changes over the life span. (After Baker 1970.)

**VADE MECUM**

A segment on gametogenesis in mammals has movies and photographs that illustrate the streamlining of the sperm and remarkable growth of the egg, taking you deeper into the mammalian gonad with each step.

**WEB TOPIC 6.10** **THE BIOCHEMISTRY OF OOCYTE MATURATION** The maturation of the oocyte is intimately connected to several hormones produced by the brain. The effects of these hormones are mediated by the follicle cells of the ovary in fascinating ways.

**OOGENIC MEIOSIS** Oogenic meiosis in mammals differs from spermatogenic meiosis not only in its timing but in the placement of the metaphase plate. When the primary oocyte divides, its nuclear envelope, breaks down, and the metaphase spindle migrates to the periphery of the cell (see Severson et al. 2016). This asymmetric cytokinesis is directed through a cytoskeletal network composed chiefly of filamentous actin that cradles the mitotic spindle and brings it to the oocyte cortex by myosin-mediated contraction (Schuh and Ellenberg 2008). At the cortex, an oocyte-specific tubulin mediates the separation of chromosomes, and mutations in this tubulin have been found to cause infertility (Feng et al. 2016). At telophase, one of the two daughter cells contains hardly any cytoplasm, while the other daughter cell retains nearly the entire volume of cellular constituents (**FIGURE 6.24**). The smaller cell becomes the **first polar body**, and the larger cell is referred to as the **secondary oocyte**.

A similar unequal cytokinesis takes place during the second division of meiosis. Most of the cytoplasm is retained by the mature egg (the ovum), and a second polar body forms but receives little more than a haploid nucleus. (In humans, the first polar body usually does not divide. It undergoes apoptosis around 20 hours after the first

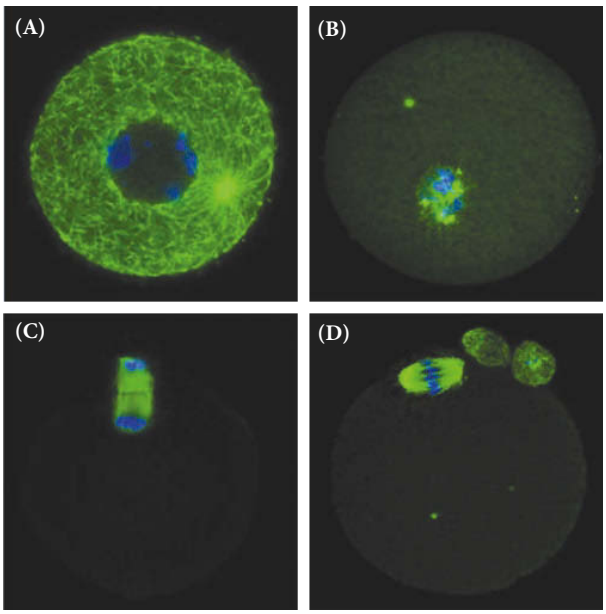



**FIGURE 6.24** Meiosis in the mouse oocyte. The tubulin of the microtubules is stained green; the DNA is stained blue. (A) Mouse oocyte in meiotic prophase. The large haploid nucleus (the germinal vesicle) is still intact. (B) The nuclear envelope of the germinal vesicle breaks down as metaphase begins. (C) Meiotic anaphase I, wherein the spindle migrates to the periphery of the egg and releases a small polar body. (D) Meiotic metaphase II, wherein the second polar body is given off (the first polar body has also divided). (From De Vos 2002, courtesy of L. De Vos.)

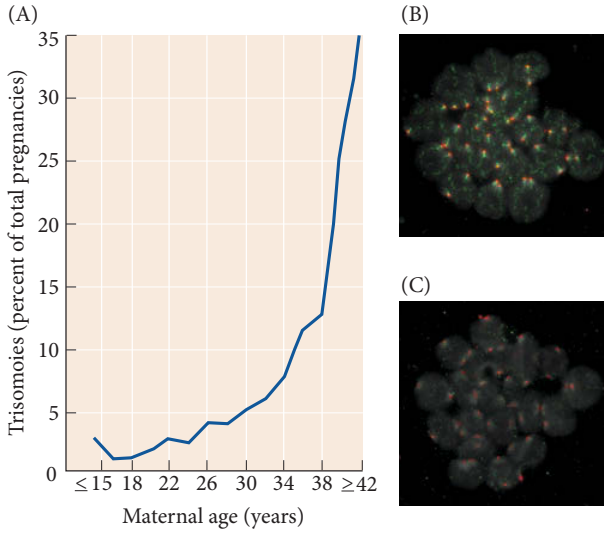



**FIGURE 6.25** Chromosomal nondisjunction and meiosis. (A) Maternal age affects the incidence of trisomies in human pregnancy. (B,C) Reduction of chromosome-associated cohesin in aged mice. DNA (white) and cohesin (green) stained in oocyte nuclei of (B) 2-month-old (young) and (C) 14-month-old (aged, for a mouse) ovaries. A significant loss of cohesin can be seen (especially around the kinetochores) in aged mice. (A after Hunt and Hassold 2010; B,C after Lister 2010.)

meiotic division.) Thus, oogenic meiosis conserves the volume of oocyte cytoplasm in a single cell rather than splitting it equally among four progeny (Longo 1997; Schmerler and Wessel 2011).

**OOCYTES AND AGE** The retention of the oocyte in the ovary for decades has profound medical implications. A large proportion, perhaps even a majority, of fertilized human eggs have too many or too few chromosomes to survive. Genetic analysis has shown that usually such **aneuploidy** (incorrect number of chromosomes) is due primarily to errors in oocyte meiosis (Hassold et al. 1984; Munné et al. 2007). Indeed, the percentage of babies born with aneuploidies increases greatly with maternal age. Women in their 20s have only a 2–3% chance of bearing a fetus whose cells contain an extra chromosome. This risk goes to 35% in women who become pregnant in their 40s (**FIGURE 6.25A**; Hassold and Chiu 1985; Hunt and Hassold 2010). The reasons for this appear to be at least twofold. The first reason concerns the breakdown of cohesin proteins (Chiang et al. 2010; Lister et al. 2010; Revenkova et al. 2010). Once made and assembled, cohesins remain on the chromosomes for decades, but they are gradually lost as the cell ages (**FIGURE 6.25B,C**). This loss of protein and function is accelerated as the cells become physiologically senescent. The second reasons concerns the fact that human meiotic metaphase is remarkably long (16 hours to assemble a meiotic spindle in humans compared to 4 hours in mice) and the linkage between the kinetochore and the spindle does not seem very stable (Holubcová et al. 2015).

## Coda

Thus the sex-determining mechanisms have assembled either ovaries or testes, and their respective gametes, the egg and sperm, have been made. When the sperm and egg are released from their gonads, they are cells on the verge of death. However, if they meet, an organism with a lifespan of decades can be generated. The stage is now set for one of the greatest dramas of the cycle of life—fertilization.

Nature has many variations on her masterpiece. In some species, including most mammals and insects, sex is determined by chromosomes; in other species, sex is a matter of environmental conditions. In yet other species, both environmental and genotypic sex determination can function, often in different geographical areas. Different environmental or genetic stimuli may trigger sex determination through a series of conserved pathways. As Crews and Bull (2009) have reflected, "it is possible that

the developmental decision of male versus female does not flow through a single gene but is instead determined by a 'parliamentary' system involving networks of genes that have simultaneous inputs to several components of the downstream cascade." We are finally beginning to understand the mechanisms by which this "masterpiece of nature" is created.

## Next Step Investigation

Our knowledge of sex determination and gametogenesis is remarkably incomplete. First, we know very little about the fundamental processes of meiosis, namely, homologue pairing and how chromosomes are separated at first meiotic metaphase. These are processes fundamental to genetics, development, and evolution, yet we know little about them. We also need to know a great deal more about the cellular and tissue-level processes of gonad formation. We know many of the genes involved, but we are still relatively ignorant about how the testes form such that the germ cells are inside the organ and the ovaries form with their germ cells on the outside. And, of course, the relationship of developmental biology to sexual behaviors is in its infancy.

### Closing Thoughts on the Opening Photo

**This hermaphroditic chicken is split into a male (rooster) half with coxcomb, waddle, and light coloring, and a female (hen) half with darker coloring. Half the cells are ZW and half are ZZ (recall that birds have ZW/ZZ chromosomal sex determination), probably resulting from the egg's failure to extrude a polar body during meiosis and its subsequent fertilization by a separate sperm. In chickens, each cell makes its own sexual decision. In mammals, hormones play a much larger role in making a unified phenotype, and such man/woman chimeras don't arise (see Zhao et al. 2010). (Photograph courtesy of Michael Clinton.)**

## 6 Snapshot Summary
## *Sex Determination and Gametogenesis*

1. In mammals, primary sex determination (the determination of gonadal sex) is a function of the sex chromosomes. XX individuals are usually females, XY individuals are usually males.
2. The mammalian Y chromosome plays a key role in male sex determination. XY and XX mammals both have a bipotential gonad. In XY animals, Sertoli cells differentiate and enclose the germ cells within testis cords. The interstitial mesenchyme generates other testicular cell types, including the testosterone-secreting Leydig cells.
3. In XX mammals, the germ cells become surrounded by follicle cells in the cortex of the gonadal rudiment. The epithelium of the follicles becomes the granulosa cells; the mesenchyme generates the thecal cells.
4. In humans, the *SRY* gene encodes the testis-determining factor on the Y chromosome. *SRY* synthesizes a nucleic acid-binding protein that functions as a transcription factor to activate the evolutionarily conserved *SOX9* gene.
5. The *SOX9* gene product can also initiate testis formation. Functioning as a transcription factor, it binds to the gene encoding anti-Müllerian hormone and other genes. Fgf9 and Sox9 proteins have a positive feedback loop that activates testicular development and suppresses ovarian development.
6. Wnt4 and Rspo1 are involved in mammalian ovary formation. These proteins upregulate production of β-catenin; the functions of β-catenin include promoting the ovarian pathway of development while blocking the testicular pathway of development. The Foxl2 transcription factor is also required and appears to act in parallel with the Wnt4/Rspo1 pathway.
7. Secondary sex determination in mammals involves the factors produced by the developing gonads. In male mammals, the Müllerian duct is destroyed by the AMH produced by the Sertoli cells, while testosterone produced by the Leydig cells enables the Wolffian duct to

differentiate into the vas deferens and seminal vesicle. In female mammals, the Wolffian duct degenerates with the lack of testosterone, whereas the Müllerian duct persists and is differentiated by estrogen into the oviducts, uterus, cervix, and upper portion of the vagina. Individuals with mutations of these hormones or their receptors may have a discordance between their primary and secondary sex characteristics.

8. The conversion of testosterone to dihydrotestosterone in the genital rudiment and prostate gland precursor enables the differentiation of the penis, scrotum, and prostate gland.
9. In *Drosophila*, sex is determined by the number of X chromosomes in the cell; the Y chromosome does not play a role in sex determination. There are no sex hormones, so each cell makes a sex-determination "decision." However, paracrine factors play important roles in forming the genital structures.
10. The *Drosophila Sex-lethal* gene is activated in females (by the accumulation of proteins encoded on the X chromosomes), but the protein does not form in males because of translational termination. Sxl protein acts as an RNA splicing factor to splice an inhibitory exon from the *transformer* (*tra*) transcript. Therefore, female flies have an active Tra protein but males do not.
11. The Tra protein also acts as an RNA splicing factor to splice exons from the *doublesex* (*dsx*) transcript. The *dsx* gene is transcribed in both XX and XY cells, but its pre-mRNA is processed to form different mRNAs, depending on whether Tra protein is present. The proteins translated from both *dsx* messages are active, and they activate or inhibit transcription of a set of genes involved in producing the sexually dimorphic traits of the fly.
12. Sex determination of the brain may have different downstream agents than in other regions of the body. *Drosophila* Tra proteins also activate the *fruitless* gene in males (but not in females); in mammals, the *Sry* gene may activate brain sexual differentiation independently from the hormonal pathways.
13. In turtles and alligators, sex is often determined by the temperature experienced by the embryo during the time of gonad determination. Because estrogen is necessary for ovary development in these species, it is possible that differing levels of aromatase (an enzyme that can convert testosterone into estrogen) distinguish male from female patterns of gonadal differentiation.
14. The precursors of the gametes are the primordial germ cells (PGCs). In most species (*C. elegans* being an exception), the PGCs form outside the gonads and migrate into the gonads during development.
15. The cytoplasm of the PGCs in many species contains inhibitors of transcription and translation, such that they are both translationally and transcriptionally silent.
16. In most organisms studied, the coordination of germline sex (sperm/egg) is coordinated to somatic sex (male/female) by signals coming from the gonad (testis/ovary).
17. In humans and mice, germ cells entering ovaries initiate meiosis while in the embryo; germ cells entering testes do not initiate meiosis until puberty.
18. The first division of meiosis separates the homologous chromosomes. The second division of meiosis splits the kinetochore and separates the chromatids.
19. Spermatogenic meiosis in mammals is characterized by the production of four gametes per meiosis and by the absence of meiotic arrest. Oogenic meiosis is characterized by the production of one gamete per meiosis and by a prolonged first meiotic prophase to allow the egg to grow.
20. In male mammals, the PGCs generate stem cells that last for the life of the organism. PGCs do not become stem cells in female mammals (although in many other animal groups, PGCs do become germ stem cells in the ovaries).
21. In female mammals, germ cells initiate meiosis and are retained in the first meiotic prophase (dictyate stage) until ovulation. In this stage, they synthesize mRNAs and proteins that will be used for gamete recognition and early development of the fertilized egg.
22. In some species, meiosis is modified such that a diploid egg is formed. Such species can produce a new generation parthenogenetically, without fertilization.

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