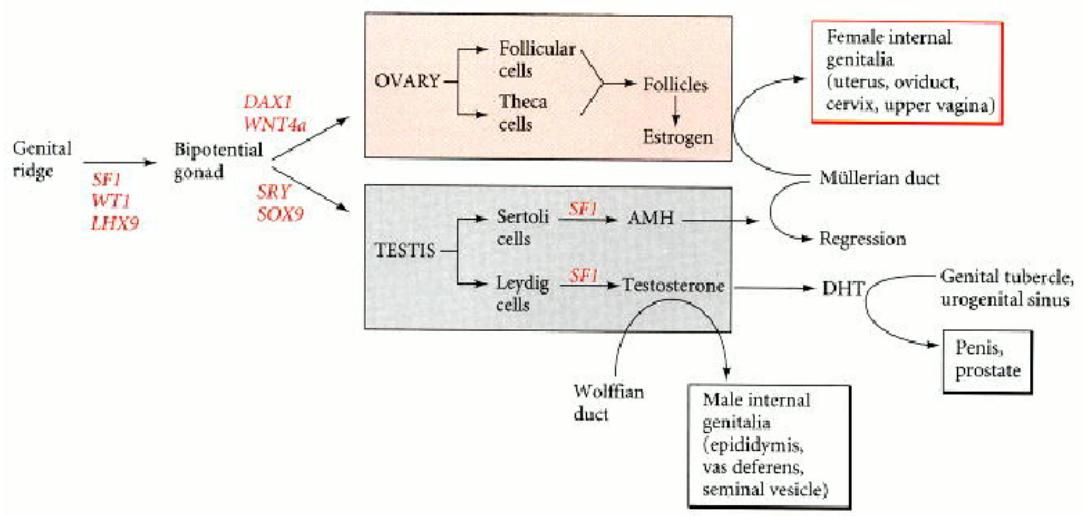
Chromosomal Sex Determination in Mammals

Primary and secondary sex determination

Primary sex determination is the determination of the gonads. In mammals, primary sex determination is strictly chromosomal and is not usually influenced by the environment. In most cases, the female is XX and the male is XY. Every individual must have at least one X chromosome. Since the female is XX, each of her eggs has a single X chromosome. The male, being XY, can generate two types of sperm: half bear the X chromosome, half the Y. If the egg receives another 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. The Y chromosome carries a gene that encodes a testis-determining factor. This factor organizes the gonad into a testis rather than an ovary. Unlike the situation in *Drosophila* (discussed below), the mammalian Y chromosome is a crucial factor for determining sex in mammals. A person with five X chromosomes and one Y chromosome (XXXXXY) would be male. Furthermore, an individual with only a single X chromosome and no second X or Y (i.e., XO) develops as a female and begins making ovaries, although the ovarian follicles cannot be maintained. For a complete ovary, a second X chromosome is needed. In mammalian primary sex determination, there is no "default state." The formation of ovaries and testes are both active, gene-directed processes. Moreover, as we shall see, both diverge from a common precursor, the bipotential gonad.

Secondary sex determination affects the bodily phenotype outside the gonads. A male mammal has a penis, seminal vesicles, and prostate gland. A female mammal has a vagina, cervix, uterus, oviducts, and mammary glands. In many species, each sex has a sex-specific size, vocal cartilage, and musculature. These secondary sex characteristics are usually determined by hormones secreted from the gonads. However, in the absence of gonads, the female phenotype is generated. When Jost (1953) removed fetal rabbit gonads before they had differentiated, the resulting rabbits had a female phenotype, regardless of whether they were XX or XY. They each had oviducts, a uterus, and a vagina, and each lacked a penis and male accessory structures.

The general scheme of mammalian sex determination is shown in Figure 17.2. If the Y chromosome is absent, the gonadal primordia develop into ovaries. The ovaries produce **estrogen**, a hormone that enables the development of the **Müllerian duct** into the uterus, oviducts, and upper end of the vagina. If the Y chromosome is present, testes form and secrete two major hormones.



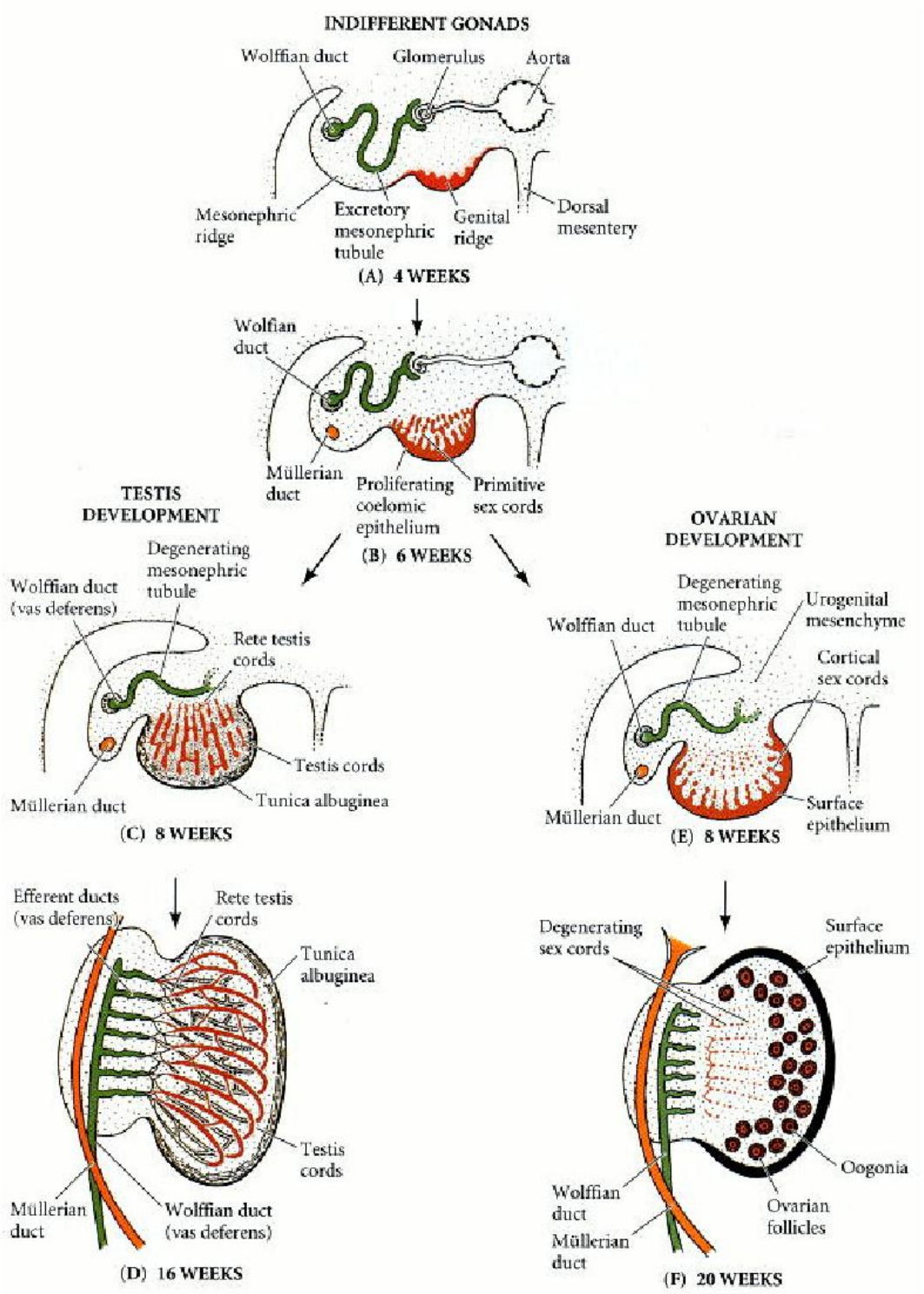
The first hormone—**anti-Müllerian duct hormone** (**AMH**; also referred to as Müllerian-inhibiting substance, MIS)—destroys the Müllerian duct. The second hormone—**testosterone**—masculinizes the fetus, stimulating the formation of the penis, scrotum, and other portions of the male anatomy, as well as inhibiting the development of the breast primordia. Thus, the body has the female phenotype unless it is changed by the two hormones secreted by the fetal testes. We will now take a more detailed look at these events.

The developing gonads

The gonads embody a unique embryological situation. All other organ rudiments can normally differentiate into only one type of organ. A lung rudiment can become only a lung, and a liver rudiment can develop only into a liver. The gonadal rudiment, however, has two normal options. When it differentiates, it can develop into either an ovary or a testis. The path of differentiation taken by this rudiment determines the future sexual development of the organism. But, before this decision is made, the mammalian gonad first develops through a **bipotential (indifferent) stage**, during which time it has neither female nor male characteristics.

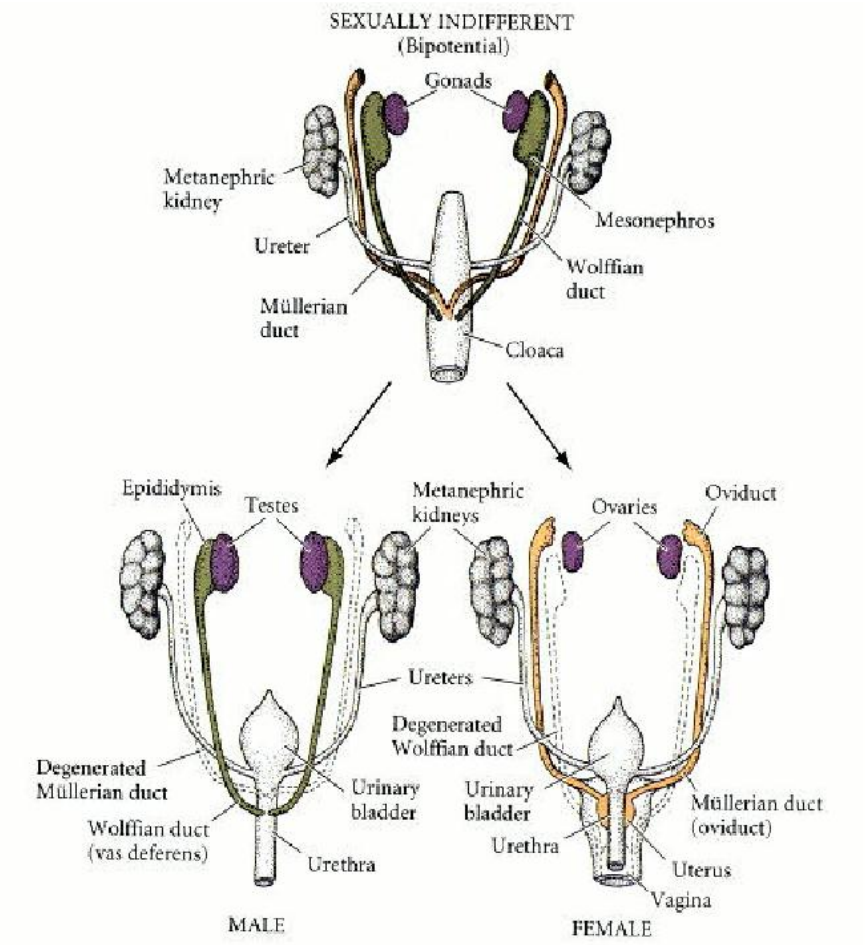
In humans, the gonadal rudiments appear in the intermediate mesoderm during week 4 and remains sexually indifferent until week 7. The gonadal rudiments are paired regions of the intermediate mesoderm; they form adjacent to the developing kidneys. The ventral portions of the gonadal rudiments are composed of the genital ridge epithelium. During the indifferent stage, the genital ridge epithelium proliferates into the loose connective mesenchymal tissue above it (Figure 17.3A,B). These epithelial layers form the **sex cords**. The germ cells migrate into the gonad during week 6, and are surrounded by the sex cords. In both XY and XX gonads, the sex cords remain connected to the surface epithelium.

If the fetus is XY, the sex cords continue to proliferate through the eighth week, extending deeply into the connective tissue. These cords fuse, forming a network of internal (medullary) sex cords and, at its most distal end, the thinner **rete testis** (Figure 17.3C,D). Eventually, the sex cords—now called **testis cords**—lose contact with the surface epithelium and become separated from it by a thick extracellular matrix, the **tunica albuginea**. Thus, the germ cells are found in the cords within the testes. During fetal life and childhood, the testis cords remain solid. At puberty, however, the cords will hollow out to form the **seminiferous tubules**, and the germ cells will begin to differentiate into sperm.



The cells of the seminiferous tubule are called **Sertoli cells**. The Sertoli cells of the testis cords nurture the sperm and secrete anti-Müllerian duct hormone. The sperm are transported from the inside of the testis through the rete testis, which joins the **efferent ducts**.

These efferent tubules are the remnants of the mesonephric kidney, and they link the testis to the Wolffian duct, which used to be the collecting tube of the mesonephric kidney (see Chapter 15). In males, the Wolffian duct differentiates to become the **epididymis** (adjacent to the testis) and the **vas deferens**, the tube through which the sperm pass into the urethra and out of the body. Meanwhile, during fetal development, the interstitial mesenchyme cells of the testes differentiate into **Leydig cells**, which make testosterone.



GONADS		
Gonadal type	Testis	Ovary
Sex cords	Medullary (internal)	Cortical (external)
DUCTS		
Remaining duct for germ cells	Wolffian	Müllerian
Duct differentiation	Vas deferens, epididymis, seminal vessicle	Oviduct, uterus, cervix, upper portion of vagina

In females, the germ cells will reside near the outer surface of the gonad. Unlike the sex cords in males, which continue their proliferation, the initial sex cords of XX gonads degenerate. However, the epithelium soon produces a new set of sex cords, which do not penetrate deeply

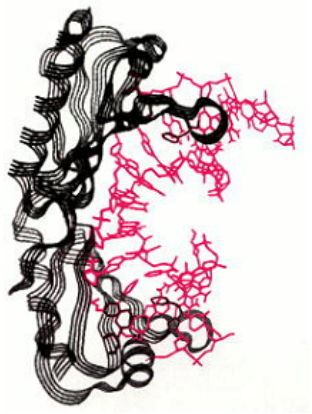
into the mesenchyme, but stay near the outer surface (cortex) of the organ. Thus, they are called **cortical sex cords**. These cords are split into clusters, with each cluster surrounding a germ cell (Figure 17.3E,F). The germ cells will become the ova, and the surrounding cortical sex cords will differentiate into the **granulosa cells**. The mesenchyme cells of the ovary differentiate into the **thecal cells**. Together, the thecal and granulosa cells will form the **follicles** that envelop the germ cells and secrete steroid hormones. Each follicle will contain a single germ cell. In females, the Müllerian duct remains intact, and it differentiates into the oviducts, uterus, cervix, and upper vagina. The Wolffian duct, deprived of testosterone, degenerates. A summary of the development of mammalian reproductive systems is shown in Figure 17.4.

The mechanisms of mammalian primary sex determination

Several genes have been found whose function is necessary for normal sexual differentiation. Unlike those that act in other developing organs, the genes involved in sex determination differ extensively between phyla, so one cannot look at *Drosophila* sex-determining genes and expect to see their homologues directing mammalian sex determination. However, since the phenotype of mutations in sex-determining genes is often sterility, clinical studies have been used to identify those genes that are active in determining whether humans become male or female. Experimental manipulations to confirm the functions of these genes can be done in mice.

Sry: the Y chromosome sex determinant

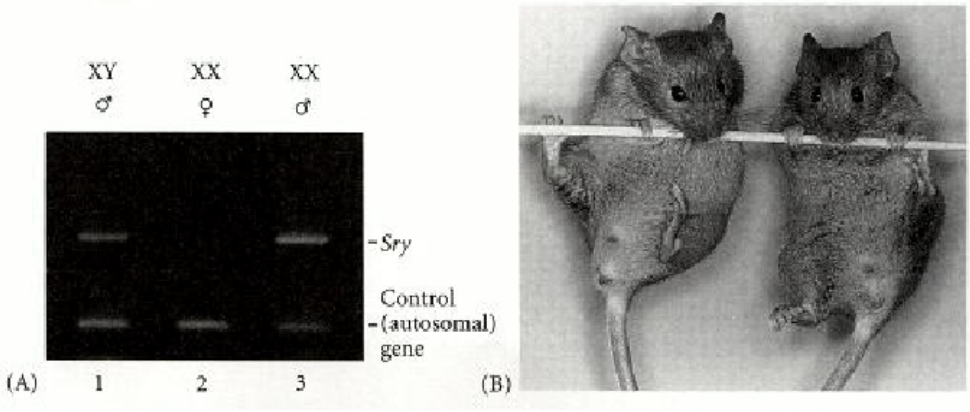
In humans, the major gene for the testis-determining factor resides on the short arm of the Y chromosome. Individuals who are born with the short arm but not the long arm of the Y chromosome are male, while individuals born with the long arm of the Y chromosome but not the short arm are female. By analyzing the DNA of rare XX men and XY women, the position of the testis-determining gene has been narrowed down to a 35,000-base-pair region of the Y chromosome located near the tip of the short arm. In this region, Sinclair and colleagues (1990) found a male-specific DNA sequence that could encode a peptide of 223 amino acids. This peptide is probably a transcription factor, since it contains a DNA-binding domain called the **HMG** (*h*igh-*m*obility *g*roup) **box**. This domain is found in several transcription factors and nonhistone chromatin proteins, and it induces bending in the region of DNA to which it binds (Figure 17.5; Giese et al. 1992). This gene is called ***SRY*** (*s*ex-determining *r*egion 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 in the rare XX males, and it is absent from normal XX females and from many XY females. Another group of XY females was found to have point or frameshift mutations in the *SRY* gene; these mutations prevent the SRY protein from binding to or bending DNA (Pontiggia et al. 1994; Werner et al. 1995). It is thought that several testis-specific genes contain SRY-binding sites in their promoters or enhancers, and that the binding of SRY to these sites begins the developmental pathway to testis formation (Cohen et al. 1994).



If *SRY* actually does encode the major testis-determining factor, one would expect that it would act in the genital ridge immediately before or during testis differentiation. This prediction has been met in studies of the homologous gene found in mice.

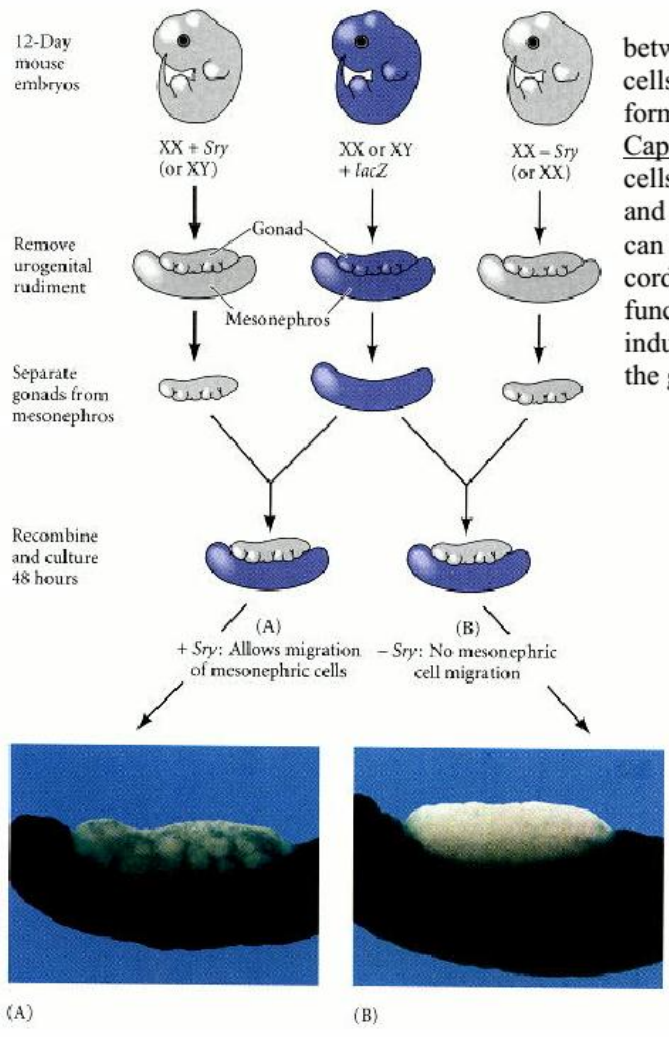
The mouse gene (*Sry*) also correlates with the presence of testes; it is present in XX males and absent in XY females (Gubbay et al. 1990; Koopman et al. 1990). The *Sry* gene is expressed in the somatic cells of the bipotential mouse gonad immediately before or during its differentiating into a testis; its expression then disappears (Hacker et al. 1995).

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, the XX embryos injected with this sequence developed testes, male accessory organs, and penises (Figure 17.6). (Functional sperm were not formed, but they were not expected, either, because 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.) Therefore, there are good reasons to think that *Sry/SRY* is the major gene on the Y chromosome for testis determination in mammals.

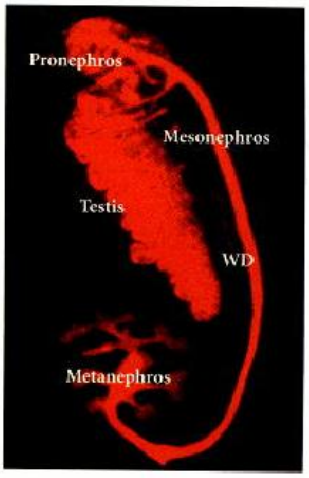


Sry/SRY is necessary, but not sufficient, for the development of the mammalian testis. Studies on mice (Eicher and Washburn 1983; Washburn and Eicher 1989; Eicher et al. 1996) have shown that the *Sry* gene of some strains of mice failed to produce testes when placed into a different strain of mouse. When the Sry protein binds to its sites on DNA, it probably creates large conformational changes. It unwinds the double helix in its vicinity and bends the DNA as much as 80 degrees (Pontiggia et al. 1994; Werner et al. 1995). This bending may bring distantly bound proteins of the transcription apparatus into close contact, enabling them to interact and influence transcription. The identities of these proteins are not yet known, but they, too, are needed for testis determination.

SRY may have more than one mode of action in converting the bipotential gonads into testes. It had been assumed for the past decade that SRY worked directly in the genital ridge to convert the epithelium into male-specific Sertoli cells. Recent studies (Capel et al. 1999), however, have suggested that SRY works via an indirect mechanism: SRY in the genital ridge cells induces the cells to secrete a chemotactic factor that permits the migration of mesonephric cells into the XY gonad. These mesonephric cells induce the gonadal epithelium to become Sertoli cells with male-specific gene expression patterns. The researchers found that when they cultured XX gonads with either XX or XY mesonephrons, the mesonephric cells did not enter the gonads. However, when they cultured XX or XY mesonephrons with XY gonads, or with gonads from XX mice containing the *Sry* transgene, the mesonephric cells did enter the gonads (Figure 17.7).



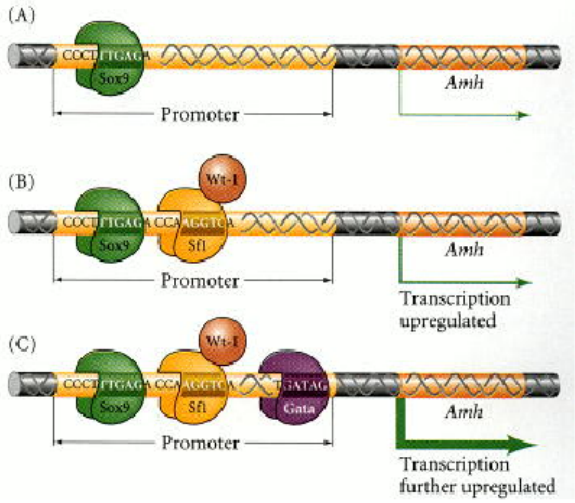
There was a strict correlation between the presence of Sry in the gonadal cells, mesonephric cell migration, and the formation of testis cords. Tilmann and Capel (1999) showed that mesonephric cells are critical for testis cord formation and that the migrating mesonephric cells can induce XX gonadal cells to form testis cords. It appears, then, that Sry may function indirectly to create testes by inducing mesonephric cell migration into the gonad.



(C)

Sox9: autosomal sex reversal

One of the autosomal genes involved in sex determination is ***SOX9***, which encodes a putative transcription factor that also contains an HMG box. XX humans who have an extra copy of *SOX9* develop as males, even though they have no *SRY* gene (Huang et al. 1999). Individuals having only one functional copy of this gene have a syndrome called campomelic dysplasia, a disease involving numerous skeletal and organ systems. About 75% of XY patients with this syndrome develop as phenotypic females or hermaphrodites (Foster et al. 1994; Wagner et al. 1994; Mansour et al. 1995). It appears that *SOX9* is essential for testis formation. The mouse homologue of this gene, *Sox9,* is expressed only in male (XY) but not in female (XX) genital ridges. Moreover, *Sox9* expression is seen in the same genital ridge cells as *Sry,* and it is expressed just slightly after *Sry* expression (Wright et al. 1995; Kent et al. 1996). The Sox9 protein binds to a promoter site on the *Amh* gene, providing a critical link in the pathway toward a male phenotype (Figure 17.8; Arango et al. 1999). While *Sry* is found specifically in mammals, *Sox9* is found throughout the vertebrates. *Sox9* may be the older and more central sex determination gene, although in mammals it became activated by its relative, *Sry*.

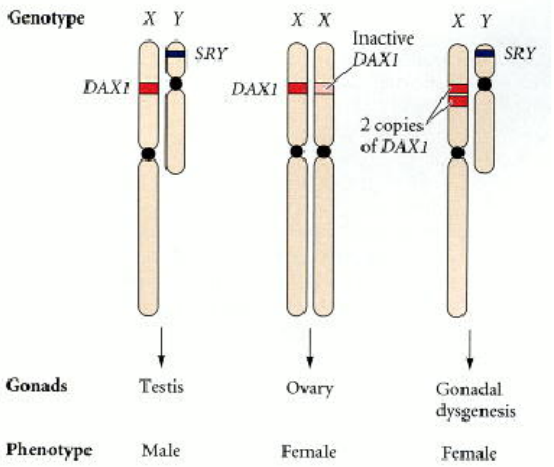


Sf1: the link between sry and the male developmental pathways

Another protein that may be directly or indirectly activated by SRY is the transcription factor **SF1** (*s*teroidogenic *f*actor *1*). *Sf1* is necessary to make the bipotential gonad; but while Sf1 levels decline in the genital ridge of XX mouse embryos, the *Sf1* gene stays on in the developing testis. Sf1 appears to be active in masculinizing both the Leydig and the Sertoli cells.

In the Sertoli cells, Sf1, working in collaboration with Sox9, is needed to elevate the levels of AMH transcription (see Figure 17.8; Shen et al. 1994; Arango et al. 1999). In the Leydig cells, Sf1 activates the genes encoding the enzymes that make testosterone. The importance of SF1 for testis development and AMH regulation in humans is demonstrated by an XY patient who is heterozygous for *SF1*. Although the genes for SRY and SOX9 are normal, this individual has malformed fibrous gonads and retains fully developed Müllerian duct structures (Achermann et al. 1999). It is thought that SRY (directly or indirectly) activates the *SF1* gene, and the SF1 protein then activates both components of the male sexual differentiation pathway (Sertoli AMH and Leydig testosterone).

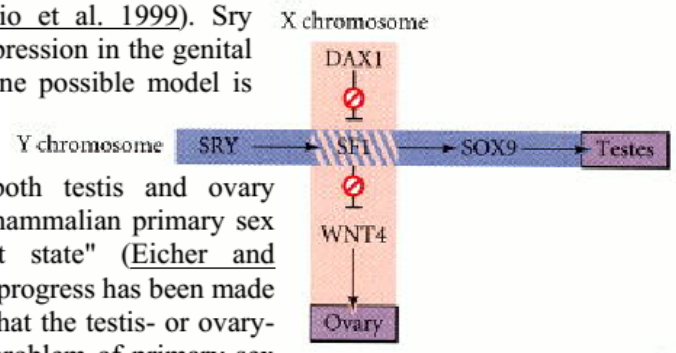
Dax1: a potential ovary-determining gene on the X chromosome



In 1980, Bernstein and her colleagues reported two sisters who were genetically XY. Their Y chromosomes were normal, but they had a duplication of a small portion of the short arm of the X chromosome. Subsequent cases were found, and it was concluded that if there were two copies of this region on the active X chromosome, the SRY signal would be reversed (Figure 17.9). Bardoni and her colleagues (1994) proposed that this region contains a gene for a protein that competes with the SRY factor and that is important in directing the development of the ovary. In testicular development, this gene would be suppressed, but having two active copies of the gene would override this suppression. This gene, ***DAX1***, has been cloned and shown to encode a member of the nuclear hormone receptor family (Muscatelli et al. 1994; Zanaria 1994). *Dax1* is expressed in the genital ridges of the mouse embryo, shortly after *Sry* expression. Indeed, in XY mice, *Sry* and *Dax1* are expressed in the same cells. DAX1 appears to antagonize the function of SRY, and it down-regulates SF1 expression (Nachtigal et al. 1998; Swain et al. 1998). Thus, *DAX1* is probably a gene that is involved in ovary determination.

Wnt4: a potential ovary-determining gene on an autosome

The ***WNT4*** gene is another gene that may be critical in ovary determination. This gene is expressed in the mouse genital ridge while it is still in its bipotential stage. *Wnt4* expression then becomes undetectable in XY gonads (which become testes), whereas it is maintained in XX gonads as they begin to form ovaries. In transgenic XX mice that lack the *Wnt4* genes, the ovary fails to form properly, and its cells express testis-specific markers, including AMH- and testosterone-producing enzymes (Vainio et al. 1999). Sry may form testes by repressing *Wnt4* expression in the genital ridge, as well as by promoting *Sf1*. One possible model is shown in Figure 17.10.



It should be realized that both testis and ovary development are active processes. In mammalian primary sex determination, neither is a "default state" (Eicher and Washburn 1986). Although remarkable progress has been made in recent years, we still do not know what the testis- or ovary-determining genes are doing, and the problem of primary sex determination remains (as it has since prehistory) one of the great unsolved problems of biology.

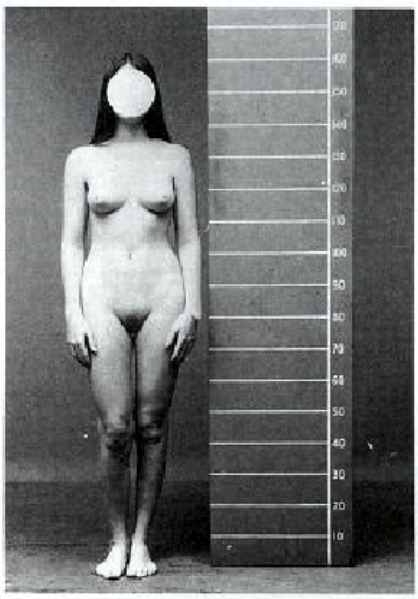
Secondary sex determination: Hormonal regulation of the sexual phenotype

Primary sex determination involves the formation of either an ovary or a testis from the bipotential gonad. This, however, does not give the complete sexual phenotype. Secondary sex determination in mammals involves 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 occurs within the embryo during organogenesis; the second occurs during adolescence.

As mentioned earlier, if the bipotential gonads are removed from an embryonic mammal, the female phenotype is realized: the Müllerian ducts develop while the Wolffian duct degenerates. This pattern also is seen in certain humans who are born without functional gonads. Individuals whose cells have only one X chromosome (and no Y chromosome) originally develop ovaries, but these ovaries atrophy before birth, and the germ cells die before puberty. However, under the influence of estrogen, derived first from the ovary but then from the mother and placenta, these infants are born with a female genital tract (Langman and Wilson 1982).

The formation of the male phenotype involves the secretion of two testicular hormones. The first of these hormones is AMH, the hormone made by the Sertoli cells that causes the degeneration of the Müllerian duct. The second is the steroid testosterone, which is secreted from the fetal Leydig cells. This hormone causes the Wolffian duct to differentiate into the epididymis, vas deferens, and seminal vesicles, and it causes the urogenital swellings to develop into the scrotum and penis.

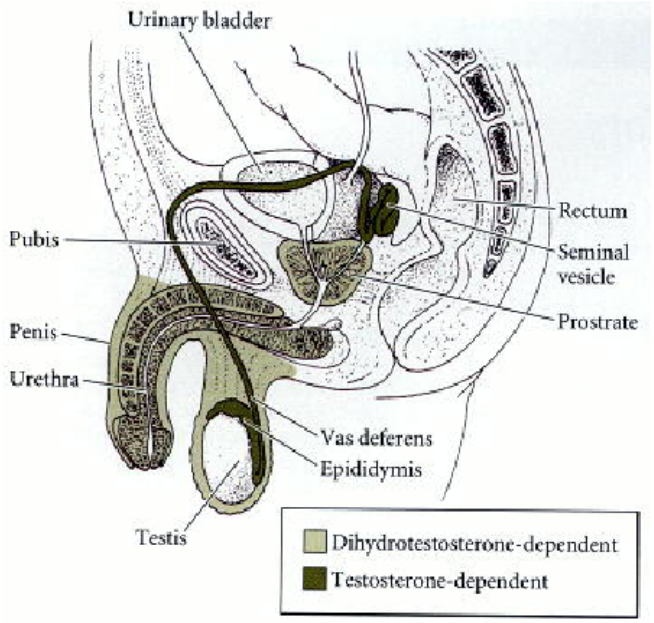
The existence of these two independent systems of masculinization is demonstrated by people having **androgen insensitivity syndrome**. These XY individuals have the *SRY* gene, and thus have testes that make testosterone and AMH. However, they lack the testosterone receptor protein, and therefore cannot *respond* to the testosterone made by their testes (Meyer et al. 1975). Because they are able to respond to estrogen made in their adrenal glands, they develop the female phenotype (Figure 17.11).



However, despite their distinctly female appearance, these individuals do have testes, and even though they cannot respond to testosterone, they produce and respond to AMH. Thus, their Müllerian ducts degenerate. These people develop as normal but sterile women,* lacking a uterus and oviducts and having testes in the abdomen.

Testosterone and dihydrotestosterone

Although testosterone is one of the two primary masculinizing hormones, there is evidence that it might not be the active masculinizing hormone in certain tissues. Testosterone appears to be responsible for promoting the formation of the male reproductive structures (the epididymis, seminal vesicles, and vas deferens) that develop from the Wolffian duct primordium.

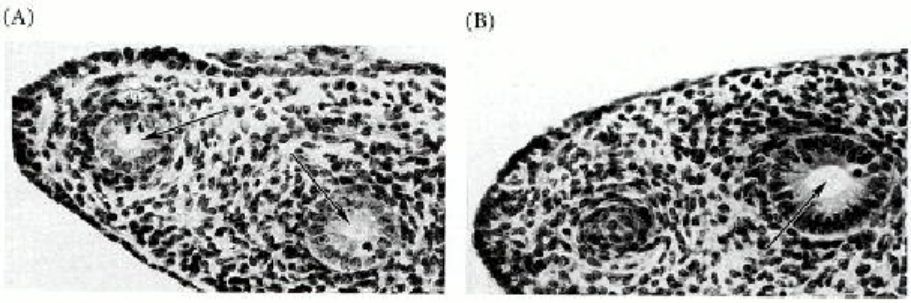


However, it does not directly masculinize the male urethra, prostate, penis, or scrotum. These latter functions are controlled by **5a-dihydrotestosterone** (Figure 17.12). Siiteri and Wilson (1974) showed that testosterone is converted to 5□-dihydrotestosterone in the urogenital sinus and swellings, but not in the Wolffian duct. 5□-dihydrotestosterone appears to be a more potent hormone than testosterone.

The importance of 5□-dihydrotestosterone was demonstrated by Imperato-McGinley and her colleagues (1974). They found a small community in the Dominican Republic in which several inhabitants had a genetic deficiency of the enzyme 5□-ketosteroid reductase 2, the enzyme that converts testosterone to dihydrotestosterone. These individuals lack a functional gene for this enzyme (Andersson et al. 1991; Thigpen et al. 1992). Although XY children with this syndrome have functioning testes, they have a blind vaginal pouch and an enlarged clitoris. They appear to be girls and are raised as such. Their internal anatomy, however, is male: they have testes, Wolffian duct development, and Müllerian duct degeneration. Thus, it appears that the formation of the external genitalia is under the control of dihydrotestosterone, whereas Wolffian duct differentiation is controlled by testosterone itself. Interestingly, when the testes of these children produce more testosterone at puberty, the external genitalia are able to respond to the higher levels of the hormone, and they differentiate. The penis enlarges, the scrotum descends, and the person originally thought to be a girl is shown to be a young man.

Anti-müllerian duct hormone

Anti-Müllerian duct hormone (AMH), the hormone that causes the degeneration of the Müllerian duct, is a 560-amino acid glycoprotein secreted from the Sertoli cells (Tran et al. 1977; Cate et al. 1986). When fragments of fetal testes or isolated Sertoli cells are placed adjacent to cultured tissue segments containing portions of the Wolffian and Müllerian ducts, the Müllerian duct atrophies even though no change occurs in the Wolffian duct (Figure 17.13). AMH is thought to bind to the mesenchyme cells surrounding the Müllerian duct and to cause these cells to secrete a paracrine factor that induces apoptosis in the Müllerian duct epithelium (Trelstad et al. 1982; Roberts et al. 1999).



Estrogen

Estrogen is needed for the complete development of both the Müllerian and the Wolffian ducts. In females, estrogen secreted from the fetal ovaries appears sufficient to induce the differentiation of the Müllerian duct into its various components: the uterus, oviducts, and cervix. The extreme sensitivity of the Müllerian duct to estrogenic compounds is demonstrated by the teratogenic effects of **diethylstilbesterol** (**DES**), a powerful synthetic estrogen that can cause infertility by changing the patterning of the Müllerian duct (see Mittendorf 1995). In mice, DES can cause the oviduct epithelium to take on the appearance of the uterus, and the uterine epithelium to resemble that of the cervix (Ma et al. 1998).

In males, estrogen is actually needed for fertility. One of the functions of the efferent duct (vas efferens) cells is to absorb about 90% of the water from the lumen of the rete testis. This concentrates the sperm, giving them a longer lifespan and providing more sperm per ejaculate. This absorption of water is regulated by estrogen. If estrogen or its receptor is absent in mice, this water is not absorbed, and the mouse is sterile (Hess et al. 1997). While blood concentrations of estrogen are higher in females than in males, the concentration of estrogen in the rete testis is even higher than that in female blood.

*Androgen insensitivity syndrome is one of several conditions called *pseudohermaphroditism.* In a pseudohermaphrodite, there is only one type of gonad, but the secondary sex characteristics differ from what would be expected from the gonadal sex. In humans, male pseudohermaphroditism can be caused by mutations in the androgen receptor or by mutations affecting testosterone synthesis (Geissler et al. 1994). Female pseudohermaphroditism can be caused by an overproduction of testosterone.

True hermaphrodites (rare in humans, but the norm in some invertebrates such as nematodes and earthworms) contain both male and female gonadal tissue. Mammalian true hermaphrodites result from abnormalities of primary sex determination. Such abnormalities can occur when the Y chromosome is translocated to the X chromosome. In those tissues where the translocated X chromosome is inactivated during dosage compensation, the *SRY* gene will be turned off. However, in those tissues where the translocated X chromosome is not inactivated, the *SRY* gene will be on (Berkovitz et al. 1992; Margarit et al. 2000).

Sex Determination and Behaviors

Organization/Activation Hypothesis

Does prenatal (or neonatal) exposure to particular steroid hormones impose permanent sex-specific changes on the central nervous system? Such sex-specific neural changes have been shown in regions of the brain that regulate "involuntary" sexual physiology. The cyclic secretion of luteinizing hormone by the adult female rat pituitary, for example, is dependent on the lack of testosterone during the first week of the animal's life. The luteinizing hormone secretion of female rats can be made noncyclic by giving them testosterone 4 days after birth; conversely, the luteinizing hormone secretion of males can be made cyclical by removing their testes within a day of birth (Barraclough and Gorski 1962). It is thought that sex hormones may act during the fetal or neonatal stage of a mammal's life to organize the nervous system in a sex-specific manner, and that during adult life, the same hormones may have transitory, activational effects. This idea is called the **organization/activation hypothesis.**

Interestingly, the hormone chiefly responsible for determining the male brain pattern is **estradiol**, a type of estrogen.* Testosterone in fetal or neonatal blood can be converted into estradiol by the enzyme P450 aromatase, and this conversion occurs in the hypothalamus and limbic system—two areas of the brain known to regulate hormone secretion and reproductive behavior (Reddy et al. 1974; McEwen et al. 1977). Thus, testosterone exerts its effects on the nervous system by being converted into estradiol. But the fetal environment is rich in estrogens from the gonads and placenta. What stops these estrogens from masculinizing the nervous system of a female fetus? Fetal estrogen (in both males and females) is bound by **a-fetoprotein**. This protein is made in the fetal liver and becomes a major component of the fetal blood and cerebrospinal fluid. It will bind and inactivate estrogen, but not testosterone.

Attempts to extend the organization/activation hypothesis to "voluntary" sexual behaviors are more controversial because there is no truly sex-specific behavior that distinguishes the two sexes of many mammals, and because hormonal treatment has multiple effects on the developing mammal. For instance, injecting testosterone into a week-old female rat will increase pelvic thrusting behavior and diminish lordosis—a posture that stimulates mounting behavior in the male—when she reaches adulthood (Phoenix et al. 1959; Kandel et al. 1995). These behavioral changes can be ascribed to testosterone-mediated changes in the central nervous system, but they could also be due to hormonal effects on other tissues. Testosterone enables the growth of the muscles that allow pelvic thrusting. And since testosterone causes females to grow larger and to close their vaginal orifices, one cannot conclude that the lack of lordosis is due solely to testosterone-mediated changes in the neural circuitry (Harris and Levine 1965; De Jonge et al. 1988; Moore 1990; Moore et al. 1992; Fausto-Sterling 1995).

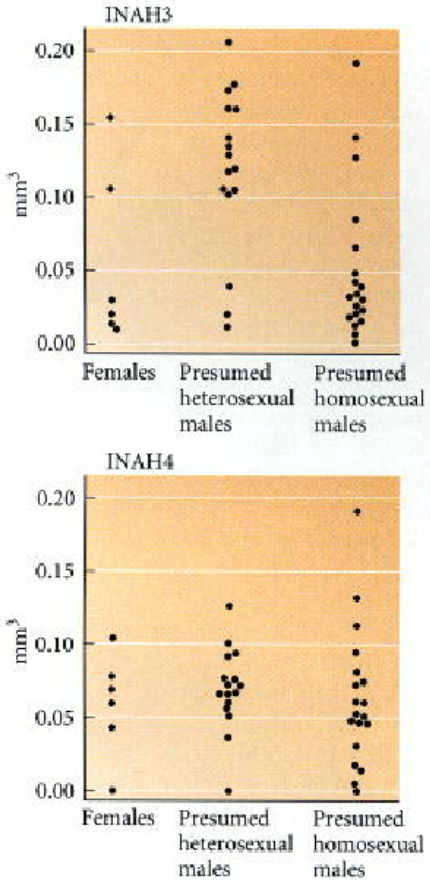
In addition, the effects of sex steroids on the brain are very complicated, and the steroids may be metabolized differently in different regions of the brain. Male mice lacking the testosterone receptor still retain a male-specific preoptic morphology in the brain, and male mice lacking the aromatase enzyme are capable of breeding (Breedlove 1992; Fisher et al. 1998). These studies show that there is more to sex-specific morphology and behavior than steroid hormones. Despite best-selling books that pretend to know the answers, we have much more to learn regarding the relationship between development, steroids, and behavior. Moreover, extrapolating from rats to humans is a very risky business, as no sex-specific behavior has yet been identified in humans, and what is "masculine" in one culture may be considered "feminine"

in another (see Jacklin 1981; Bleier 1984; Fausto-Sterling 1992). As one review (Kandel et al. 1995) concludes:

There is ample evidence that the neural organization of reproductive behaviors, while importantly influenced by hormonal events during a critical prenatal period, does not exert an immutable influence over adult sexual behavior or even over an individual's sexual orientation. Within the life of an individual, religious, social, or psychological motives can prompt biologically similar persons to diverge widely in their sexual activities.

Male Homosexuality

Certain behaviors are often said to be part of the "complete" male or female phenotype. The brain of a mature man is said to be formed such that it causes him to desire mating with a mature woman, and the brain of a mature woman causes her to desire to mate with a mature man. However, as important as desires are in our lives, they cannot be detected by in situ hybridization or isolated by monoclonal antibodies. We do not yet know if sexual desires are primarily instilled in us by our social education or are fundamentally "hardwired" into our brains by genes or hormones during our intrauterine development or by other means.



In 1991, Simon LeVay proposed that part of the anterior hypothalamus of homosexual men has the anatomical form typical of women rather than of heterosexual men. The hypothalamus is thought to be the source of our sexual urges, and rats have a sexually dimorphic area in their anterior hypothalamus that appears to regulate their sexual behavior. Thus, this study generated a great deal of publicity and discussion. The major results are shown in Figure 17.14. The interstitial nuclei (neuron clusters) of the anterior hypothalamus (INAH) were divided into four regions. Three of them showed no signs of sexual dimorphism. However, one of them, INAH3, showed a statistically significant difference in volume between males and females; it was claimed that the male INAH3 is, on average, more than twice as large as the female INAH3. Moreover, LeVay's data suggested that the INAH3 of homosexual men was similar in volume to that of women and less than half the size of heterosexual men's INAH3. This finding, LeVay claimed, "suggests that sexual orientation has a biological substrate."

There have been several criticisms of LeVay's interpretation of the data. First, the data are from populations, not individuals. One can also say that there is a statistical range and that men and women have the same general range.

Indeed, one of the INAH3 from a homosexual male was larger than all but one of those from the 16 "heterosexual males" in the study. Second, the "heterosexual men" were not necessarily heterosexual, nor were the "homosexual men" necessarily homosexual; the brains came from corpses of people whose sexual preferences were not known.

This brings up another issue: homosexuality has many forms, and is probably not a single phenotype. Third, the brains of the "homosexual men" were taken from patients who had died of AIDS. AIDS affects the brain, and its effect on the hypothalamic neurons is not known.

Fourth, because the study was done on the brains of dead subjects, one cannot infer cause and effect. Such data show only correlations, not causation. It is as likely that behaviors can affect regional neuronal density as it is that regional neuronal density can affect behaviors. If one interprets the data as indicating that the INAH3 of male homosexuals is smaller than that of male heterosexuals, one still does not know whether that is a cause of homosexuality or a result of it. Indeed, Breedlove (1997) has shown that the density and size of certain neurons in rat spinal ganglia depend on the frequency of sexual intercourse. In this case, the behavior was affecting the neurons. Fifth, even if a difference in INAH3 does exist, there is no evidence that the difference has anything to do with sexuality. Sixth, these studies do not indicate when such differences (if they exist) emerge. The question of whether differences among the heterosexual male, female, and homosexual male INAH3 occur during embryonic development, shortly after birth, during the first few years of life, during adolescence, or at some other time was not addressed.

In 1993, a correlation was made between a particular DNA sequence on the X chromosome and a particular subgroup of male homosexuals: homosexual men who had a homosexual brother. Out of 40 pairs of homosexual brothers wherein one brother had inherited a particular region of the X chromosome from his mother, the other brother had also inherited this region in 33 cases (Hamer et al. 1993). One would have expected both brothers to have done so in only 20 cases, on average. Again, this is only a statistical concordance, and one that could be coincidental. Moreover, the control (the incidence of the same marker in the "nonhomosexual" males of these families) was not reported, and the statistical bias of the observations has been called into question, especially since other laboratories have not been able to repeat the result (Risch et al. 1993; Marshall 1995). More recent studies (Hu et al. 1995; Rice et al. 1999) found little or no increase in the incidence of this DNA sequence when homosexual men were compared to their nonhomosexual brothers. Hu and colleagues concluded that this sequence is "neither necessary nor sufficient for a homosexual orientation." Thus, despite the reports of these studies in the public media, no "gay gene" has been found.

Genes encode RNAs and proteins, not behaviors. While genes may bias behavioral outcomes, we have no evidence for their "controlling" them. The observance of people with schizophrenia, or people whose personalities change radically after a religious conversion or a traumatic experience, indicates that a single genotype can support a wide range of personalities. This is certainly a problem with any definition of a "homosexual phenotype," since people can alternate between homosexual and heterosexual behavior, and the definition of what is homosexual behavior differs between cultures (see Carroll and Wolpe 1996). Thus, whether homosexual desires are formed by genes within the nucleus, by sex hormones during fetal development, or by experiences after birth is still an open question.

*The terms *estrogen* and *estradiol* are often used interchangeably. However, estrogen refers to a class of steroid hormones responsible for establishing and maintaining specific female characteristics. Estradiol is one of these hormones, and in most mammals (including humans), it is the most potent of the estrogens.