 American Journal of Medical Genetics Part A 143A:3054–3068 (2007)

Research Review

Genetics of Sexual Development: A New Paradigm

Stan R. Blecher[1]* and Robert P. Erickson[2,3]

[1]Department of Molecular Biology and Genetics (Emeritus), University of Guelph, Guelph, Ontario, Canada
[2]Department of Pediatrics, College of Medicine, University of Arizona, Tucson, Arizona
[3]Department of Molecular and Cellular Biology, College of Medicine, University of Arizona, Tucson, Arizona

Received 25 April 2007; Accepted 6 August 2007

The classical paradigm of human and mammalian sexual development, largely based on work of Alfred Jost, depicts the genetic factor(s) that determine(s) sex as influencing only the fate of the gonad. A maleness factor produces testes (*Primary Sex Determination*). These organs secrete hormones which cause male *Secondary Sexual Differentiation*. In absence of the maleness factor, by default the gonad becomes an ovary, and the absence of testicular hormones leads to female secondary differentiation. In this article a new paradigm is proposed, to accommodate recent findings. Sexual dimorphism precedes gonadal development, in a *Pregonadal Stage*. Furthermore, female development is not by default—both male (Y) and female (X) sex-chromosomal primary sex-determining mechanisms probably exist. The human/mammalian male Y-chromosomal sex-determining gene is now known (*SRY/Sry*), and a candidate for a non-inactivated, X-linked, female determining factor, is under study. However, the proximate gonad-determining genes are probably on autosomes. Pathways between the primary factors and the proximate gonad-determining genes are indirect and complex. A hypothetical gene Z has been proposed, that inhibits the testis determiner and is itself the target of suppression by *SRY/Sry*. Candidates for proximate testis and ovary-determining factors and for *Z* also exist. The "default" concept has also been superseded with respect to secondary sexual differentiation. Absence of testicular hormones does not produce a normal female phenotype; ovarian genes and hormones are necessary. Finally, sex-chromosomal sex-determining genes influence the development not only of non-gonadal organs of secondary sexual development, but also of organs outside of the reproductive system. © 2007 Wiley-Liss, Inc.

Key words: sexual development; Jost; testis-determining factor; ovary; secondary differentiation; *SRY*; epididymis; scrotum; penoscrotal transposition

How to cite this article: Blecher SR, Erickson RP. 2007. Genetics of sexual development: A new paradigm. Am J Med Genet Part A 143A:3054–3068.

INTRODUCTION

Since the middle of the last century our understanding of sexual development of humans, and of mammals in general, has been based on the classical work of such researchers as Witschi [1948, 1956], Gillman [1948] and, in particular, on principles enunciated by Alfred Jost. The latter performed ground-breaking experimental research, mainly on the rabbit [reviewed in Jost et al., 1973]. The importance of this work was so substantial that to this day its major precepts are still valid. However, subsequent research has revealed new data that modify the conclusions of Jost, and which now legitimately claim a place in our understanding of the field. In the following we first outline the tenets of the classical paradigm, widely known as Jost's Principle, and then describe the various research results that drive the requirement for a change in paradigm.

JOST'S PRINCIPLE

Jost performed surgical castration on fetal rabbits, and found that irrespective of the genetic sex of the

Grant sponsor: Institute for the Study of Man in Africa; Grant sponsor: Kalahari Research Committee (South Africa); Grant sponsor: Danish Research Foundation (Denmark); Grant sponsors: Medical Research Council and the Natural Sciences and Engineering Research Council (Canada).

*Correspondence to: Stan R. Blecher, 141 Janefield Avenue, Guelph, Ontario, Canada N1G 2L4. E-mail: sblecher@uoguelph.ca

DOI 10.1002/ajmg.a.32037

fetus, in the absence of the gonad, secondary sexual development (i.e., development of the internal duct systems and the external genitalia) proceeded in a female pattern. That is, the Wolffian duct system (K.F. Wolff, 1733–1794), which normally disappears in females but in males develops into the epididymis and vas deferens (Table I), regressed in both sexes in the castrated fetuses. The Mullerian duct (Johannes Muller, 1801–1858), which regresses in males but is the normal precursor of the uterine tubes, the uterus and part of the vagina, persisted in both male and female experimental animals. Externally, the labioscrotal folds formed labia and the genital tubercle a clitoris. In other experiments, testosterone was injected into female fetuses, and male-type development of both external genitalia and the Wolffian duct ensued. From this Jost brilliantly concluded that the testis promoted masculine secondary development by producing hormones which supported growth of internal and external male structures, and other hormone molecules which suppressed female organs.

Subsequent work by others, including Nathalie Josso [reviewed in Josso et al., 2006], expanded on the original formulation of Jost, and led to the generally accepted framework of sexual development. Jost's Principle can be stated as follows: at fertilization, in the first stage of dimorphic sexual development, known as primary sex determination, sex is assigned by the presence, in males, or the absence, in females, of a genetic factor or factors that determine(s) the fate of the gonad. Notwithstanding this assignment of sex at conception, in early ontogeny the entire reproductive system exists in a basic, unspecialized state, identical in both sexes and hence known as the ''indifferent'' stage, and no dimorphic effects of the initial sex determination occur till about 6 weeks in humans. At that stage the sex-determining genetic factor, when present (i.e., in males) causes the hitherto indifferent gonad to develop as a testis, and in the factor's absence, in females, the gonad becomes an ovary. Thus the testis represents the induced state and the ovary the default. Shortly after it becomes morphologically identifiable as such, the testis commences secretion of two types of hormones. First, the androgens, testosterone and dihydrotestosterone, promote Wolffian duct development and cause the external precursor structures to masculinize. Second, Mullerian Inhibiting Substance (MIS) suppresses the development of the paramesonephric ducts. In this stage, known as secondary sexual differentiation, the development of the male internal organs, such as the epididymis and vas deferens, and the external structures (penis and scrotum), are again perceived as representing the induced state. The absence of testosterone and hence of the male organs, and the failure of the Mullerian tube to regress, represent the uninduced, default state in the female. Genetic sex determination is exclusively associated with the fate of the gonad, and all other sexual development is the domain of the hormones. By corollary, sex determining genes are not involved in, and do not influence, any development other than that of the gonads, and the testicular hormones are essential and sufficient causation of all secondary sexual development.

A POST-JOST PARADIGM

Although the above formulation of Jost's hypothesis mentions ''a genetic factor or factors,'' at the time very little was known about the nature of the genetic control of sexual development in humans or other mammals. Early research in genetics, at the beginning of the 20th century, was mainly done using *Drosophila* as the model, and the sex-determining mechanism in that genus had been shown to be dependent on a balance between X chromosomes and autosomes, and to not involve the Y. It was assumed that the same would pertain to humans. It was not until the late1950s that the first major step in elucidating mammalian sexual development occurred. Since then, much research has provided new information on several aspects of sexual development that was not envisaged by Jost. This includes the chromosomal basis of sex determination in mammals, early (embryonic and fetal) sexual dimorphism, intermediary gene pathways, genetic induction of both testis and ovary, and effects of sex chromosomal and sex determining

TABLE I. Embryological Origins of the Major Components of the Reproductive System

	Fate	
Precursor	Male	Female
Gonad	Testis	Ovary
Internal duct systems		
Mesonephric (Wolffian)	Epididymis, vas deferens	Mainly regresses
Paramesonephric (Mullerian)	Mainly regresses	Uterine tubes, uterus and upper part of vagina
Urogenital sinus	(Urethra)	Lower part of vagina (and urethra)
External genitalia		
Genital tubercle	Penis	Clitoris
Labioscrotal folds	Scrotum	Labia

factors on secondary sexual development and that of other organs.

THE CORE GENETIC FACTORS OF SEXUAL DIMORPHISM: BOTH SEX CHROMOSOMES DO DETERMINE SEX IN MAMMALS

Although it had been known since 1923 that humans have both X and Y chromosomes [Painter, 1923] it was only in 1959 that evidence from three different studies established that in humans and most other mammals, unlike *Drosophila*, the Y chromosome is sex determining. Patients with Ullrich–Turner syndrome, who have a basically female phenotype, were shown to have a 45,X sex karyotype [Ford et al., 1959] while Klinefelter syndrome, with male genitalia, was characterized by XXY [Jacobs and Strong, 1959]. Mice of X0 karyotype are both female in phenotype and fertile [Welshons and Russell, 1959]. The Y chromosome was hence considered to be the bearer of a factor that was both necessary and sufficient for male sex-determination, independent of the presence of X chromosomes. These data were also interpreted to confirm the concept, now also at the sex-chromosomal level, of the male inducer and, in its absence, the female default state. The hypothetical male-inducing gene on the Y was named testis-determining factor (*TDF*) in humans, and testis determiner on the Y (*Tdy*) in mice. The search for *TDF* began in earnest.

The primary region of homology, synapsis and crossing over of the human sex chromosomes, now known as the pseudoautosomal region, is situated at the tips of the p (short) arms of the X and Y. (A second such region also exists at the tips of the q (long) arms.) Evans et al. [1979] had shown that XX humans of male phenotype often have a translocated segment of Yp on the Xp arm. This finding suggested that the situation of *TDF* might be close to the proximal border of the p pseudoautosomal region, and that occasional incorrect alignment in meiotic synapsis could lead to anomalous cross-over, resulting in translocation of *TDF* to an X. However, at that time molecular techniques were not available to pursue this finding.

Many candidates for the hypothetical *TDF* gene were explored in the succeeding years. Early, apparently promising candidates included *H-Y*, *Bkm* and *Zfy*. *HY* (histocompatibility antigen on the Y), a marker identified in skin transplantation experiments in mice, was favored because of its ubiquitous and evolutionarily conserved presence in many species [Wachtel et al., 1975]. However, this gene was excluded when phenotypically male XX*Sxr* mice that were HY negative [McLaren et al., 1984], and HY positive human XY individuals of female phenotype [Simpson et al., 1987] were reported. *Bkm* (banded krait minor), a repetitive sequence isolated from a snake, was reported to be present only on sex chromosomes in some vertebrates [Jones and Singh, 1981]. Erickson and co-workers showed, though, that in the human karyotype *Bkm* was widely dispersed and not specifically located in the sex specific region of the Y [Kiel-Metzger et al., 1985].

By the late 1980s molecular techniques had made possible the construction of deletion maps of the sex-determining region of the human Y, from study of XX persons of male phenotype due to anomalous X-Y cross-over [Vergnaud et al., 1986]. Page et al. [1987] identified a gene they named zinc finger gene on the Y (*ZFY*), in the stretch of Y adjacent to the pseudoautosomal region, which they claimed was *TDF*. However, some XX males they studied did not have *ZFY*, indicating that this claim was erroneous. Renewed study of XX males who lacked *ZFY* revealed a new gene, named sex-determining region on the Y (*SRY*), in the region of the Y between the proximal border of the pseudoautosomal region and the translocational breakpoints in such males [Sinclair et al., 1990]. *SRY* contains one conserved sequence homologous to the mating-type protein of a yeast, and another conserved sequence homologous to a DNA-binding motif of high mobility group (HMG) transcriptional proteins. The mouse homologue of *SRY*, *Sry*, was isolated at the same time [Gubbay et al., 1990]. The evidence supporting *SRY/Sry* as the key maleness genes on the Y chromosome includes the fact that they are localized in the smallest maleness-determining region of the Y [Gubbay et al., 1990; Sinclair et al., 1990], and *Sry* has been experimentally shown to induce masculine development in transgenic mice [Koopman et al., 1991]. Thus *SRY/Sry* and their homologues in other mammals are believed to be the true Y-chromosomal male-determining factor though, as will be described below, they are evidently not the immediate testis-inducing genes.

As mentioned, the discovery of the male-inducing role of the mammalian and human Y chromosome in 1959, deduced from the phenotypes of Ullrich–Turner and Klinefelter syndromes, appeared to confirm the concept of the male as the induced state. At the time less attention was paid to the fact that in Ullrich–Turner syndrome the phenotype is not that of a normal female, nor in Klinefelter syndrome that of a normal male.

In Klinefelter syndrome (XXY), testes are present and the genitalia are of male type. On this basis it is argued that the presence of a single Y overrides the effects of even more than one X chromosome; even XXXXY individuals have testes. However, in Klinefelter syndrome (XXY) patients, as well as in XXXXY, the testes are very abnormal and the phenotype is partially feminized. The latter may include female bodily habitus and body-hair distribution, gynecomastia, and feminine pitch of the voice. The mechanism of this feminization is not known, but it seems evident that it must be due to the presence of a

significant level of estrogens, presumably produced by Sertoli cells, which share a common origin with ovarian follicle cells [Gillman, 1948]; (see later). A non-inactivated X locus and incomplete Lyonization of X chromosomes may result in the presence of more than one active copy of an X-chromosomal gene that directs the development of these estrogen-producing cells. In Ullrich–Turner syndrome (45,X) the phenotype, though "basically" female, in fact in fundamental ways is not normal female. The external genitalia are infantile, normal breast development is lacking and, most importantly, though ovaries with oogonia are present in fetal stages, in adults the ovaries are absent and replaced by "streak gonads." This suggests that two copies of a non-inactivated X-chromosomal factor are required for normal post-natal development of the ovary. We return to this below.

Bernstein et al. [1980] reported two siblings of karyotype Xdup(p21)Y, both of whom had ovarian tissue and female external genitalia. The authors suggested the possibility of regulatory elements on the X that might suppress Y-chromosomal testis-determining genes. Subsequent authors have supported the suggestion that an X-linked gene could be involved in XY sex-reversal through the mechanism of suppression of the Y-linked *TDF*. Scherer et al. [1989] considered that *ZFX*, the X homologue of *ZFY*, which is non-inactivated, may be a candidate. Bardoni et al. [1994] suggested that a gene, named dosage sensitive sex-reversal (*DSS*) may exist in this region of Xp. *DAX-1* (*DSS*, adrenal hypoplasia congenita, on X) is a candidate for *DSS*. *Dax-1* is expressed in the developing ovary and downregulated in the testis [Swain et al., 1996], and its overexpression in transgenic animals delays testicular development and can result in sex reversal, that is, in this case, the presence of ovaries in a genetic male [Swain et al., 1998]. In XXY patients the locus must be partially inactivated in respect of *SRY* suppression, as these individuals present a predominantly male phenotype, though with abnormal testes and gynecomastia. Sexual ambiguity or reversal in 69, XXY triploids may be due to failure of X-inactivation [Petit et al., 1992]. The target of *SRY* may have been found recently in *R-spondin 1* [Parma et al., 2006]. Absence of *R-spondin 1* leads to XX sex reversal (testes in a genetic female), as if the proximate testis determining gene, now believed to be *SOX-9* (see below) is not inhibited, as it should be in female gonads.

Interestingly, in the reports cited here, and other such cases, the existence of ovarian tissue has almost invariably been interpreted in terms of the "male as induced sex, female as default" concept. But the evidence has been available since 1959, reinforced by the data from such cases as those reported by Bernstein et al. [1980], that the Y does not completely override X chromosomes (or X loci), and indeed that at least in some cases, one or more than one X-chromosomal locus can also override the Y. Furthermore, the "default" situation of absence of Y does not produce normal ovaries in the absence of a second X. In hindsight it is now clear that the normal female phenotype is not a default state caused by absence of *TDF*, but rather a status that requires the presence of two functional copies of at least one X chromosomal gene. In other words, $X \neq XX$. The data cited above provide strong evidence of a female-determining, non-inactivated locus on the normal X.

THE PREGONADAL STAGE OF SEXUAL DIMORPHISM AND DEVELOPMENT

In the classical paradigm two stages were described. The first, primary sex determination—that is, the establishment of the sex of the gonad—was envisaged as being initiated at conception, when the embryo either acquired a testis determining factor or failed to do so. Following a latent period of several weeks, in which no sexually dimorphic features were apparent in the embryo, the "indifferent" gonad manifested its morphological commitment as a testis or, by default, an ovary. Then later, when the testis commenced secreting hormones (or the ovary failed to do so) the second stage, secondary sexual differentiation, commenced.

More recent research has revealed, though, that sexually dimorphic transcripts and traits do indeed exist long before the sixth week of human development, when the gonad's sex becomes visible. Some of these may be triggering factors, or expressions of such, in the cascade of sexual development. We accordingly previously proposed [Blecher and Erickson, 2007] that a description of the sequence of events should include a pregonadal stage. Below we discuss some of the important pregonadal events of sexual development. These include early transcription of the Y-chromosomal genes *SRY*, *ZFY*, and *HY*; function of genes upstream of *SRY/Sry*; other genes that influence gonadal precursor tissue; and sexually dimorphic events for which the genes are not known.

Y-Chromosomal Genes

Erickson and co-workers and others have shown that transcription of both *SRY* and *ZFY* can be detected at the one-cell stage [Ao et al., 1994; Fiddler et al., 1995], and that in the mouse transcripts of *Sry* and *Zfy* are found at the blastocyst stage [Zwingman et al., 1993]. It is possible that these genes trigger the start of the sex determination cascade. The male specific *HY* gene, mentioned above as an erstwhile candidate for *TDF*, may also be transcribed early. A putative serologically detectable form of HY antigen, now known as serologically detectable male antigen (SMA) was shown to be expressed at the 8-cell stage in mouse embryos [Epstein et al., 1980]. The function of HY antigen is unknown.

Genes Upstream of *SRY/Sry*

The gene for alpha-thalassemia, retardation, on the X (ATR-X) syndrome, which includes sexual ambiguity, interacts with heterochromatin proteins and modifies DNA structure [Gibbons et al., 1995]. Knockout (KO) of the mouse gene *M33*, a homologue of the *Drosophila* chromatin-regulating gene *Polycomb*, causes failure of gonadal development and partial or complete male to female sex reversal [Katoh-Fukui et al., 1998]. These findings suggest that chromatin modification may initiate function of *SRY/Sry*. Fibroblast growth factor 9 (*Fgf-9*) may also be a mediator of *Sry*. KO of *Fgf-9* frequently causes sex reversal in mice [Colvin et al., 2001] as does KO of insulin receptor genes [Nef et al., 2003].

Genes Affecting Gonadal Precursor Tissues

Genes of the *Lhx* (LIM homeobox) family, acting early in development, are essential for normal development of gonads. KO of both *Lhx-1* [Shawlot and Behringer, 1995] and of Lhx-9 [Birk et al., 2000] leads to absence of gonads. *Fgf-8* is upstream of *Lhx-1* and *Lhx-9* itself is upstream of steroidogenic factor 1 (*Sf-1*), KO of which leads to absence of gonads and adrenal glands, and neonatal death. *SF-1/Sf-1*, the human and mouse homologues of the *Drosophila gene* Fushi tarazu, are expressed in the urogenital ridge. Erickson [1999] predicted that mutations in *SF-1* would result in a human syndrome comparable to that of the mouse, with female phenotype and lack of steroid hormones. The first such patient was reported by Achermann et al. [1999].

The mouse gene *Dmrt* is also expressed in the genital ridge, predominantly in the male, prior to testis differentiation. This gene and the human *DMRT-1* and *DMRT-2* are homologues of a gene shared by *Drosophila* and *C. elegans* (the genes doublesex, *dsx*, and *mab3*, respectively, from which the name *DM r*elated *t*ranscript derives) [Raymond et al., 1999]. *DMRT-1* and *DMRT-2* map to distal 9p [Raymond et al., 1999], a chromosomal region deletion of which has long been associated with testicular dysgenesis and female phenotype in 46,XY. This condition is sometimes referred to as XY sex reversal, but since ovaries are not present it would be more correctly described as a form of male pseudohermaphroditism (see below). More recently a Z-chromosome linked homologue of *DMRT* in the chicken has been shown to act as a recessive testis determining factor [Shan et al., 2000]. In the fish medaka, which has an XX/XY sex-determining system, *dmrt1Y* is a candidate male sex-determining gene, though the gene is not present in other fish [Kondo et al., 2003], and a homologue of *DMRT* is a testis determining factor in at least one reptile [Smith et al., 1999]. Thus the DM domain [Raymond et al., 1999] is clearly a very highly conserved, male-determining genomic region.

The Wilms tumor gene 1 (*Wt-1*) plays a role in testis development. The WAGR syndrome (Wilms tumor, aniridia, genitourinary abnormalities and retardation) is associated with deletions of chromosomal band 11p13, which includes the *Wt-1* gene. Aside from tumor suppressor function, the gene is evidently concerned with mesenchymal-epithelial interactions, possibly between the Leydig and Sertoli cells of the testis [Van Heyningen and Hastie, 1992]. Point mutations in *Wt-1* can result in Denys–Drash syndrome, which causes dysgenetic gonads and ambiguous external genitalia in XY individuals. The Denys–Drash variant Frasier syndrome produces partial or complete feminization of the phenotype in XY children.

Pregonadal Sexual Dimorphism for Which the Genes Remain Unknown

The cleavage divisions of in vitro fertilized and cultured bovine embryos were found by Yadav et al. [1993] to occur significantly earlier in male embryos than female. Scott and Holson [1977] found that in rat fetuses at 12.5 days post coitus, males were larger than females, and had higher protein content, and this faster rate of cell division has been confirmed in humans [Pergament et al., 1994]. Burgoyne [1993] reported that the Y had an accelerating effect on mouse preimplantation embryos, suggesting the possibility of a Y-linked growth factor. Thornhill and Burgoyne [1993] found that the paternal X had a retarding effect in post-implantation female mouse embryos. Peippo and Bredbacka [1995] reported that at day 3, female mouse embryos were more advanced than males, and in subsequent in vitro culture cell increase was greater in males, suggesting that reported increased rates in males might be artifacts of in vitro culture. This "Y growth factor" effect could be mediated by insulin and its receptors, since male to female sex reversal occurs in triple KO of three insulin receptor genes in mice [Nef et al., 2003]. Mittwoch [1993] proposed that rapid growth rate itself might be the cause of testis formation.

In the Tammar wallaby, a marsupial, the scrotum, gubernaculum, and processus vaginalis, and the mammary glands of the female, appear before development of the gonads and independently of hormones [O et al., 1988]. These organs develop mainly under hormonal influence in eutherian mammals, but a possible example of evolutionary conservation of genic control of scrotal space development will be mentioned below. A balance of sex chromosomal:autosomal effects in this case is also possible.

INTERMEDIARY PATHWAYS BETWEEN CHROMOSOMAL SEX DETERMINATION AND GONAD-INDUCING GENES

Although now accepted as being the Y-chromosomal male determining factor, *SRY/Sry* is not essential

for testis induction (see below). Furthermore, *SRY/Sry* does not directly act on *SOX-9/Sox-9*, the accepted candidate for the ultimate testis inducing gene (see below). These facts have led to the hypothesis that the testis inducing gene is suppressed by a hypothetical gene *Z*, which in turn itself is subject to repression by *SRY* [McElreavy et al., 1993]. Testis determination in humans in fact now appears to be the outcome of a fairly complex network of gene interactions downstream of *SRY*. Here we summarize some of the important components of this scheme, the major elements of which are depicted diagrammatically in Figure 1.

McElreavy et al. [1993] proposed that gene *Z* might be on Xp21 which, as mentioned above, is the location of duplications in some cases of 46,XY sex reversal. Thus duplication of the Xp region of interest might involve duplication of a suppressor (*Z*) of the testis-inducing gene. We mentioned above that the duplicated region may involve duplication of a female-determining gene. It is possible that one gene may serve both functions (first, *SRY* suppression and second, initiation of the femaleness pathway) or

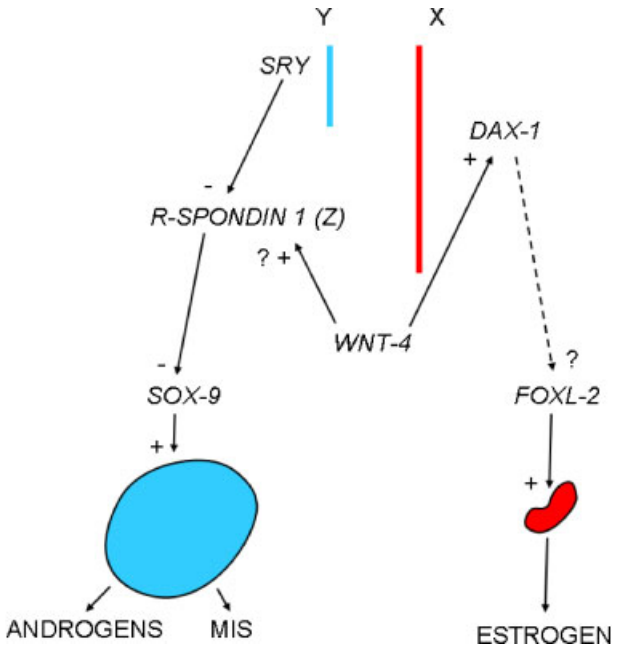

FIG. 1. Simplified, schematic overview of the genetics of sexual development. Sex-determining genes for male and female development are situated on the Y and X chromosomes, respectively. *SRY* is the male determining gene on the human Y. It represses the function of a postulated gene Z, which in turn suppresses the testis-determining factor. *SOX-9* is the major candidate for the latter, while *R-SPONDIN 1* is a recent and compelling candidate for Z. The testis secretes Androgens and Mullerian Inhibiting Substance, which control secondary sexual differentiation in the male. The absence of these hormones is required for normal female development. *DAX-1* is a likely candidate for an X-linked female determining gene, while recent research on the Polled Intersex Syndrome in goats suggests that *FOXL-2* is the proximate ovary-determining gene. The pathway of interaction between the X-linked femaleness gene and the ovary-determining factor remains to be established. Ovarian production of estrogen is essential for normal female secondary differentiation. The gene *WNT-4* appears to influence both testicular and ovarian development. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

that two genes, one for each function, may be present in this chromosomal band. Graves [1998] has proposed *SOX-3* as a candidate for a *Z*-like gene. *SOX-3* is on Xq and therefore distinct from the region of the X, and thus also from the putative female-determining locus, discussed above. As mentioned above, a new and excellent candidate for the suppressor target of *SRY* (which when derepressed activates SOX-9) is *R-spondin 1* [Parma et al., 2006].

WNT-4, which maps to the distal tip of chromosome 1p, appears to influence both testicular and ovarian development. Duplication of this gene can cause male pseudohermaphroditism [Garcia-Heras et al., 1999], and overexpression of it (in transfection experiments, in Sertoli–Leydig cell cultures) causes upregulation of *DAX-1* [Jordan et al., 2001]. KO of *Wnt-4* leaves male mice unaffected and females masculinized [Vainio et al., 1999]. Lack of *Wnt-4* causes masculinization of XX gonads and compromises Sertoli cell differentiation [Jeays-Ward et al., 2004].

DEVELOPMENTAL GENETICS OF THE GONADS

The indifferent gonad arises in the genital ridge, and three cellular components participate in specific gonadal differentiation [Gillman, 1948]. The mesenchyme of the ridge itself will provide the Leydig (androgen-producing) cells of the testis or the theca cells of the ovary. The celomic epithelium covering the ridge invaginates to become the Sertoli cells that line the seminiferous tubules, or ovarian follicular cells, and primordial germ cells migrate from the region of the developing gut, between the two layers of the mesentery, to become sperm or ova [Witschi, 1948].

Genetics of Testicular Development

The male-determining factor on the human (and mammalian) Y chromosome is unequivocally *SRY/Sry* (and their homologues), but this gene is neither necessary nor sufficient for testis induction. Thus in the strictest sense *SRY*, the human maleness-determining gene on the Y, is not the testis determining factor. The evidence that *SRY/Sry* does not directly induce the testis is that some XX true hermaphrodites do not bear *SRY* or any other Y-chromosomal sequence [Ramsay et al., 1988]. Also, the males of certain species of voles and other mammals develop testes without this gene [Just et al., 1995; Sotou et al., 2001].

A strong candidate for the ultimate testis-inducing gene is *SOX-9/Sox-9* (*SRY* HMG-box-related gene 9). Evidence supporting the candidacy of *SOX-9* as testis inducer includes data on the condition of campto-melic syndrome, caused by haploinsufficiency of *SOX-9*. XY individuals with this condition show partial sex reversal [Tommmerup et al., 1993].

Duplication of *SOX-9* was associated with masculinization in an XX fetus [Huang et al., 1999], and mouse XX *Sox-9* transgenics develop as apparent males [Vidal et al., 2001], as do female mice that overexpress *Sox-9* [Bishop et al., 2000]. *Sox-9* is only expressed in Sertoli cell precursors [Sekido et al., 2004], and KO of this gene leads to XY sex reversal [Barrionuevo et al., 2006].

Genetics of Ovarian Development

As discussed above, the classical concept of female development being the default outcome of absence of sex-chromosomal male-determining genes is obsolete. There is now evidence that initiation of ovarian development is not merely due to absence of testicular induction. Nef et al. [2005] compared microarrays of XX and XY gonadal cells and reported early, ovary-specific expression of several genes. More specifically, recent study of the Polled Intersex Syndrome (PIS) in goats, reviewed in Baron et al. [2005], has been productive on this front.

PIS produces female to male sex reversal in polled (hornless) goats. Paradoxically, the sex reversal trait behaves as a genetic recessive, whereas the hornlessness is dominant. Pailhoux et al. [2001] reported that a deletion in goat chromosome 1q43 affects a cis-regulating gene that controls at least two contiguous adjacent genes: *PIS*-regulated transcript 1 (*PISRT-1*) and *FOXL-2*. The latter is an ovary-inducing gene. In goat fetuses, *PISRT-1* and *FOXL-2* transcripts appear in higher concentration in female gonads than male. In addition to being a prerequisite for *FOXL-2* expression, *PISRT-1* also exhibits the *Z*-like feature of inhibiting *SOX-9*, and thus suppressing testis formation. In fetal gonads of both homozygotes and heterozygotes for the PIS deletion, expression of both *PISRT-1* and *FOXL-2* is suppressed. Since these two genes are respectively testicular inhibitor and ovarian inducer, this accounts for the sex-reversal in polled goats. Above we considered data relating to whether suppression of maleness and induction of femaleness are functions of one or of two separate genes.

FOXL-2 is highly conserved in vertebrates; goat chromosomal band 1q43 is homologous to human 3q23. In humans, mutation of *FOXL-2* causes blepharophimosis-ptosis-epicanthus inversus syndrome (BPES), a feature of which is ovarian failure. FOXL-2 protein has been detected in the nuclei of granulosa cells in human fetuses, but not in testes [Crisponi et al., 2001]. Thus the candidacy of this gene as an ovarian inducer is strong. That an ovary-determining factor would function through fetal granulosa (follicle) cells is not surprising; the essential role of these cells in support of normal maturation of ova has been known since the work of Ohno and Smith [1964]. We suggested (above) the possibility of a non-inactivated X-chromosomal female-determining gene. We are unaware of any research that establishes pathways connecting such a locus with *FOXL-2*, but we predict this will be forthcoming.

SECONDARY SEXUAL DIFFERENTIATION

According to the classical paradigm this stage of reproductive development is driven by the production of hormones in the male, or their absence in the female. Bipotential, indifferent precursors of external genitalia and the internal duct systems are initially present in both sexes (Table I). Shortly after it becomes morphologically recognizable, the testis initiates masculinization of both external and internal structures. Externally, under the influence of testosterone from the Leydig cells, the labioscrotal folds fuse to form the scrotum, the genital tubercle becomes the penis and, later, the testes descend into the scrotum. Internally, testosterone promotes the development of the Wolffian ducts into the epididymides, vasa deferentia and related structures, while MIS, from the Sertoli cells, causes regression of the Mullerian tubes. In females, in the absence of testosterone, the labioscrotal folds do not fuse, and form the labia, the genital tubercle forms the clitoris and the ovaries do not descend. The absence of MIS allows the Mullerian tubes to persist, and to develop into the uterine tubes and, fusing in the midline, the uterus and upper part of the vagina.

As we mentioned previously, the "basic female" phenotype that results from absence of testicular hormones is not identical to that of a normal female. In individuals with Ullrich–Turner syndrome, in whom ovaries are absent, external genitalia are "infantile" in appearance and breast development is lacking. Ovarian estrogen is required for normal development of both external sex organs and breasts.

In patients with pseudohermaphroditism, primary and secondary sexual development are discordant. Male pseudohermaphrodites have XY karyotype and testes but female or ambiguous external organs and internal duct derivatives. In androgen insensitivity syndrome (AIS, also known as testicular feminization; gene locus *TFM*) an X-linked mutation of the androgen receptor renders tissues insensitive to androgens. Accordingly, the external genitalia retain female appearance, female sex is assigned in infancy, and the Wolffian duct organs are absent. MIS is produced and Mullerian tube derivatives are also absent, including the upper part of the vagina which is thus shorter than normal. Failure of metabolism of testosterone leads to its conversion, by the enzyme aromatase, to large amounts of estrogen. This results in feminine fat distribution and substantial breast development, enhanced because the action of estrogen is unopposed by androgens [Griffin and Wilson, 1980], and maturation of the external

genitalia, and these patients are often only diagnosed at puberty, when menses fail to occur.

An example of female pseudohermaphroditism (with XX karyotype, female gonads and male or ambiguous secondary development) is seen in adrenogenital syndrome, in which deficiency of 21-hydroxylase leads to overproduction of adrenal androgens and subsequent masculinization of the fetus's external genitalia. Perlof et al. [1953] described a form of female pseudohermaphroditism associated with abnormalities of the internal genitalia, urinary system and gastrointestinal tract, and such cases may occur in the absence of testosterone or of *SRY* [Erickson et al., 1997]. Some cases may be part of a general disturbance of caudal development [Lubinsky, 1980]. Caudal malformations may also be part of penoscrotal transposition [MacKenzie et al., 1994], itself an abnormality of secondary differentiation (see below).

Several genes involved in secondary development have been identified. KO of the gene Sonic hedgehog (*Shh*) produces embryos with no external genitalia at day 12.5 post-conception [Haraguchi et al., 2001]. Fibroblast growth factors, specifically *Fgf-8* and *-10*, are involved upstream and downstream of *Shh* [Tanaka et al., 2005; Payson et al., 1996]. *Hox* genes are also involved in sexual differentiation. Double homozygous deficiency of *Hoxa-13* and *Hoxd-13* causes complete absence of external genitalia [Kondo et al., 1997] and in humans, mutations in *HOXA-13* cause hand-foot-genital syndrome with hypospadias and cryptorchidism [Mortlock and Innis, 1997].

EFFECTS OF SEX DETERMINING FACTORS ON ORGANS OTHER THAN GONADS

The classical paradigm predicts that the chromosomal sex-determining mechanism of mammals serves to determine the fate of the gonad only. Secondary differentiation is entirely hormone dependent, and sex-determining genes play no role in this phase of development, or in development of organs other than those of the reproductive system. There are, however, some exceptions, and possibly many more as yet unstudied.

Secondary Sexual Development

Sex reversal (*Sxr*) is a chromosomal sequence of the mouse Y that includes *Sry* and, as the result of an ancient chromosomal rearrangement, is transposed to the tip of the long arm where, in mouse, the pseudoautosomal region is situated. *Sxr* crosses over to the X chromosome in meiosis, and fertilization of ova by such X*Sxr* chromosomes produces XX*Sxr* mice which, by virtue of the contained *Sry* gene, are XX males. Their phenotype appears on superficial examination to be typically male, initially leading to the oxy-moronic term *normal* XX males, but they have several abnormalities of sexual development, and have more appropriately been called pseudomales. One of the abnormalities relates to the anogenital space, within which the scrotum develops. The length of the anogenital space (the anogenital distance, from penis or clitoris to anus) is used as a measure of masculinization: males have much larger anogenital distances than females. The distance is significantly smaller in pseudomales than in their normal, XY brothers (Fig. 2) and, of note, this difference is already present in prepubertal males—that is, prior to the pubertal androgen surge and thus not a feature of androgenization [Atkinson and Blecher, 1994].

We mentioned above that the scrotum develops independently of hormones in the Tammar wallaby. Because in marsupials the scrotum is ventral to the penis it has been argued that the findings in the wallaby are not relevant to eutherian mammals; but the scrotum is also ventral in numerous eutherian mammals including some primates. Penoscrotal transposition (Fig. 3) occurs in humans and shawl scrotum, a trait of for example Aarskog syndrome, may be a partial form of penoscrotal transposition. Blecher and co-workers [Atkinson and Blecher, 1994; MacKenzie et al., 1994] argued that genetic control of scrotal development is evolutionarily conserved in all mammals, and that the ventral scrotal position is overridden in most true mammals by an evolutionarily

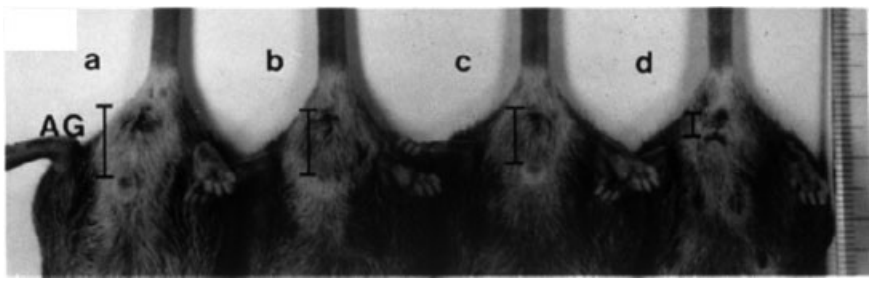

FIG. 2. Anogenital (AG) distance in mice. From left to right are illustrated the anogenital distances of a normal XY male, an X*Sxr*Y carrier, an XX*Sxr* pseudomale and a normal XX female. Females have much smaller anogenital distances than males. Pseudomales have smaller anogenital distances than normal males, and the difference is present prior to puberty. Illustration reproduced from Atkinson and Blecher [1994].

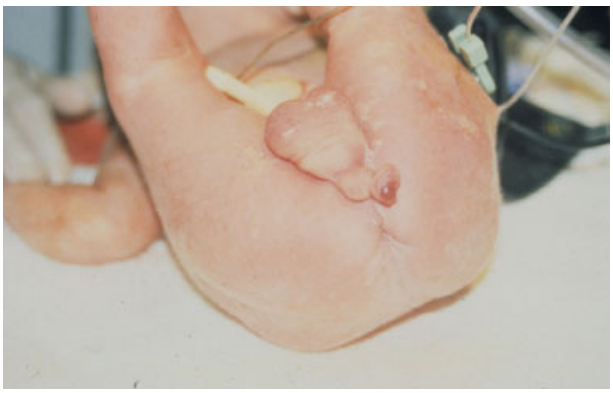

FIG. 3. Patient with penoscrotal transposition. In marsupials and some eutherian mammals including some primates, the scrotum is normally ventral to the penis. There is evidence that genes controlling scrotal development are conserved from metatherian through eutherian mammals. Penoscrotal Transposition may be an atavistic reversion to a phenotype of evolutionarily conserved genes. Illustration reproduced from MacKenzie et al. (1994). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

later innovation. Penoscrotal transposition, which resembles a homeotic mutation in Drosophila in that it produces a malpositioned organ, may be an atavistic reversion to a phenotype of existing, evolutionarily earlier genes.

Though the adult pseudomale has testosterone levels as high as or higher than the normal XY male, the epididymis lacks the important initial segment [LeBarr and Blecher, 1986] (Fig. 4) and the abnormality is present before the pubertal androgen surge [LeBarr et al., 1991], again suggesting a non-hormonally mediated effect of sex-determining genes. Abnormal "overdose" of homologous genes is known to produce maldevelopment, and in this case ancient homologues are possibly at play.

FIG. 4. Testes and epididymides of (left to right) normal male (XY), carrier (X*Sxr*Y) and pseudomale (XX*Sxr*) mice. The initial segment (arrow), an essential component of the normal mammalian epididymis, is also present in the carrier but absent in the pseudomale, though the latter has normal levels of androgens. This suggests that sex-chromosomal sex determining genes influence the development of this organ of secondary differentiation. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Pseudomales have a copy of *Sry*, which is evolved from an ancestral *Srx*, possibly the modern *Sox-3*. Two copies of that X homologue are presumably present in pseudomales (there being 2 X-chromosomes). X*Sxr*0 mice (with *Sry* and 1 X) have normal epididymides; these data suggest that the abnormality in the pseudomale is caused by the presence of three functional homologues, with the X gene of interest being non-inactivated. Also, mice with testicular feminization syndrome (*tfm*/Y males) have microscopic epididymides [Blecher, 1978], contrary to what the Jost hypothesis would predict. Thus it appears that epididymal development, and that of the anogenital space including the scrotum, are not solely dependent on testosterone, and that sex-chromosomal sex-determining loci influence these traits of secondary sexual differentiation.

The polled goat is, like the pseudomale mouse, a chromosomal female that develops testes and becomes "sex-reversed" and, as discussed above, study of the PIS [Pailhoux et al., 2001] has cast light on the genetics of ovarian development. In addition it transpires that at least one gene under the influence of the PIS cis-regulating element affects horn growth. Both *PISRT-1* and *FOXL-2* are expressed in horn buds, evidently inhibiting horn growth, in both homozygous and heterozygous mutants [Pailhoux et al., 2001]. The cis-controlling element that is deleted in PIS evidently normally inhibits this expression in the horn buds, while enhancing it in the female gonad. This elucidates the manner in which a gene of primary sex determination also affects a feature of secondary sexual maturation, independently of hormones.

Organs That Are Not Obviously Part of the Reproductive System

Anatomical sex differences—in stature, for example, and in many organs such as the skull, larynx, thorax, pelvis, and sacrum—have been known to exist for a long time, and sex hormones have been assumed to be responsible. However, in recent decades a role for sex chromosomal effects has also become evident. For example sex-linked genes for human stature [Gardiner et al., 1978] have been described. Sex-specific molecules can be isolated biochemically from many fetal animal tissues (prior to gonadal hormone secretion) including, for example, liver, kidney and spleen [Blecher et al., 1999]. It is possible that all tissues have a molecular imprint of their sex. Below we mention briefly a couple of interesting examples of possible sex-chromosomal–hormonal interactions.

In most human populations the multifactorial, quantitative genetic trait of dermatoglyphic total ridge count (TRC) of the fingers, which is established early in fetal life, is significantly higher in males than females. For example, in a large British sample Holt

[1955] found a mean for males of ~145 and for females of ~127. Penrose [1968] pointed out an inverse relationship between TRC and sex chromosomes. In Ullrich–Turner syndrome, the TRC is increased much above the mean for normal males, whereas in Klinefelter syndrome (XXY) and triple X syndrome it is much reduced. Penrose [1968] ascribed the sex-chromosomal effect to whole chromosomes or segments, rather than to sex-linked genes, with the X having a major and the Y a minor effect.

Interestingly, Polani and Polani [1979] reported that XY patients with AIS had TRC equal to control females, but that TRC was also low in the patients' fathers and mothers and other relatives. This suggests an androgen effect (in fetal life), but possibly also a pleiotropic effect of the androgen receptor gene to account for the family data. In a study of a San (African Bushman) population that had recently hybridized with Bantu-speaking Negroes, Blecher [1972] found that the sexual dimorphism of TRC was absent. There was no statistical difference between the sexes, and both sexes had counts lower than the female mean for Caucasoids. The San of both sexes show features of what classical anthropologists refer to as pedomorphism—retention of infantile features in adulthood. These include low stature, verticality of the forehead, and relative non-hirsutism of the men (Fig. 5). Females of the San and other Khoisan peoples (e.g., Hottentots) also show unusual features of sexual development. Predominant amongst these is steatopygia, an exaggeratedly feminine-type adipose deposition in the buttocks (Fig. 6). A mild form of this trait is also present in males (Fig. 5). Other enhanced features of feminization in Khoisan women include the tablier, or enlarged labia minora—the "Hottentot apron" (Fig. 7) and the globose areolar (Fig. 8). Blecher and Wilkinson [1989] proposed that these traits and others in both males and females of the San, some of which have been ascribed to pedomorphism, in fact are features of a form of feminization analogous to a partial state of AIS, possibly due to an androgen receptor that is less sensitive than in other races. This could result in the action of estrogen being partially unopposed by testosterone, as in AIS. The similarly low TRCs of the San people and of AIS patients could be thus explained by a common androgen insensitivity (or androgen receptor gene) effect. If androgens do indeed normally increase TRC (in fetal life), this effect is evidently balanced by the TRC-reducing effect of the X chromosome. The latter could possibly be due to the hypothetical X-linked feminizing locus discussed above or, in light of the Penrose "whole chromosome" hypothesis, more than one such locus: as mentioned, Klinefelter syndrome, with an extra X, is characterized both by low TRC and by feminization.

In most mammals, including humans, males have larger teeth than females [Garn et al., 1967] and this is also evident in the deciduous teeth of humans [Alvesalo and Kari, 1977]. This sexual dimorphism has been ascribed to a locus, *TSY*, on the Y chromosome [Alvesalo and de la Chapelle, 1981]. An exception is the mouse, in which male molars are smaller than female [Heller and Blecher, 1982]. By studying mouse mutants (XX*Sxr*, X0, and *Tfm*Y), in which chromosomal and hormonal sex status are discordant, the "reverse" sexual dimorphism of mouse molars was shown to be due to hormonal rather than chromosomal factors [Heller and Blecher, 1982]. The subsequent finding [Lau et al., 1989] that mice lack the Y-linked amelogenin locus, which is present in humans and possibly identical with the *TSY* locus [Salido et al., 1992], could account for this unexpected finding [Blecher,

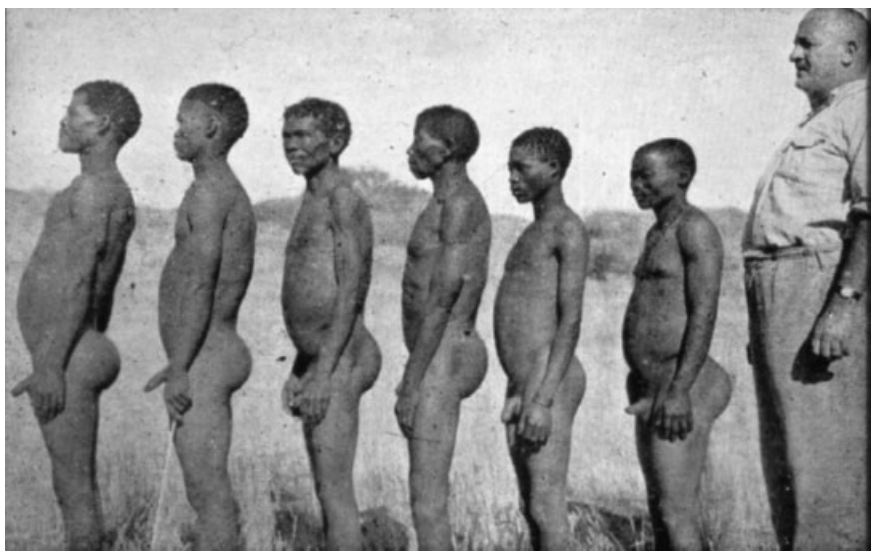

FIG. 5. San men. The men have short stature, are relatively ahirsute and have a mild degree of steatopygia. Illustration reproduced from Dart [1937].

FIG. 6. San woman. The subject illustrates the trait of steatopygia, a marked deposition of adipose tissue in the buttocks. Illustration reproduced from Dart [1937].

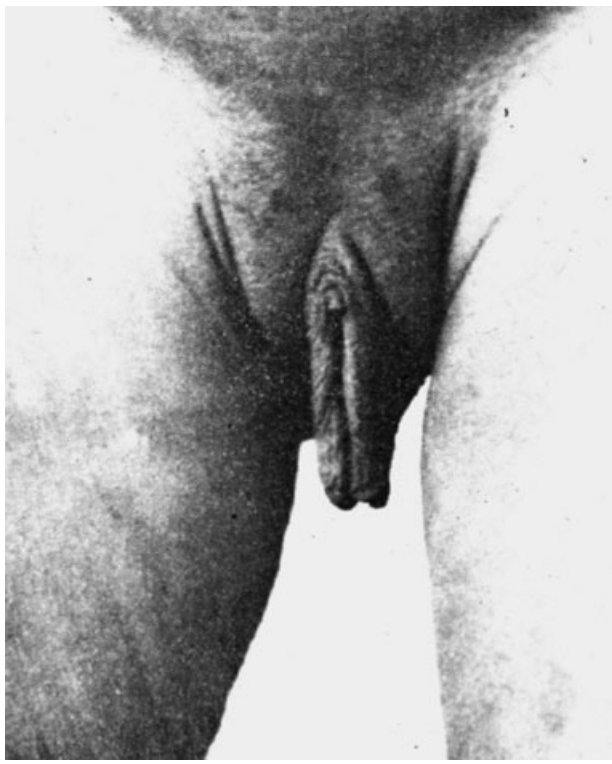

FIG. 7. Khoisan (Griqua) woman. This person demonstrates tablier (enlarged labia minora). Illustration reproduced from Orkin [1970].

1992]. This demonstrates an interesting interaction of sex-hormonal and genetic sex-determining factors in determining this trait.

Finally, the mammalian including human nervous system is also sexually dimorphic. While there is a substantial literature on the fact that sexual identity and sexual orientation are dependent on hormonal imprinting of the central nervous system in fetal life, some recent data also establish the role of cell-autonomous action of X and Y-linked genes on brain cells [reviewed in Arnold et al., 2004]. The condition of 5α-reductase deficiency is instructive. This enzyme is required for the conversion of testosterone to dihydrotestosterone, which in turn is essential for masculinization of external genitalia in fetal life. Genetically male (XY) neonates with the enzyme deficiency syndrome are diagnosed as girls. At the time of the pubertal testosterone surge the genitalia belatedly take on masculine configuration, with penile enlargement. In a study of sexual identity of 18 children with this disorder who were "unambiguously" raised as girls, 17 assumed male identity, and 16 male orientation, at puberty [Imperato-McGinley et al., 1979]. This and many other studies show that sexual identity and orientation are not learned traits, but are determined by genes, with or without fetal hormonal mediation.

Studies have shown differences in brain structure between heterosexual and homosexual men [e.g., Levay, 1991]. Recent research in Italy [Camperio-Ciani et al., 2004; Camperio-Ciani et al., in preparation] has implicated an X-chromosomal factor in human male homosexuality, and demonstrated the existence of a balanced polymorphism, with selective advantage (increased fertility) in the female carriers of this factor. This provides an answer to the "Darwinian paradox" of selective survival of genes for homosexuality. Homosexuality is widespread in natural animal populations [e.g., Bagemihl, 1999]. Studies such as those cited here and many others support the intuitive contention that genetically dimorphic sex determination and sexual reproduction are likely to be accompanied by genetically determined heterosexual attraction, and that exceptions to such attraction that occur in high (polymorphic-range) frequency are indeed due to genetic polymorphism. If this were not so, and sexual orientation were, in general, a malleable, learned or voluntary trait, species would not survive.

FIG. 8. Young San woman. The subject illustrates the trait of globose areolar. This may represent an example of robust feminization, resulting from effects of estrogen unopposed by androgens, as seen in androgen insensitivity syndrome. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

SUMMARY AND CONCLUSIONS

The classical description of the events of sexual development in humans and other mammals requires modification and updating in respect of the following points:

(1) Sex is controlled by an X-Y chromosomal mechanism. Maleness is determined by a Y-linked gene, *SRY*, in humans, or its homologue in most other mammals.
(2) However, this gene is not the immediate inducer of testis formation.
(3) Furthermore, numerous examples of pregonadal sexually dimorphic traits, at least some of which are probably due to *SRY*, develop prior to testis induction, in a Pregonadal Stage of development.
(4) For these reasons the case can be made that *SRY* is, in the strictest sense, not really the testis-determining factor, *TDF*.
(5) While the Y-linked maleness factor can, to an extent, exert its influence in the presence of two or more X chromosomes, to produce a testis, the phenotype in such cases is not that of a normal male. Thus the Y does not completely "override" the X chromosomes, evidently because of a non-inactivated X locus and incomplete Lyonization.
(6) The normal female phenotype requires two X chromosomes. The core inducer of femaleness could be an unidentified, non-inactivated X-linked gene in the region of Xp21.
(7) Two copies of this femaleness inducer can manifest their presence even in the presence of a normal Y chromosome, in some cases to even cause partial sex reversal, that is, the presence of ovarian tissue. Thus X-linked femaleness factors can, under some circumstances, also override the Y.
(8) Evidence has recently emerged, from study of the PIS in goats, for the existence of a mammalian ovary-determining gene, *Foxl2*. The human homologue, *FOXL2*, has also been identified.
(9) Testicular fetal hormones are necessary for development of normal male phenotype but are not sufficient, since in the presence of a female karyotype (two X chromosomes), abnormal development ensues even with high levels of the male hormones.
(10) The ovarian hormone estrogen is required for normal female secondary development.
(11) Thus the classical concept of the male being the induced and the female the non-induced state does not apply either at the level of primary or of secondary sexual development.
(12) Finally, the hypothesis that sex-determining factors are exclusively involved in determining the fate of the gonad, and have no influence on secondary differentiation of reproductive organs, or other (non-sex) organs, is no longer tenable. Many, and possibly all tissues bear the imprint of their genetic sex.

ACKNOWLEDGMENTS

We gratefully acknowledge the insightful comments and advice we received from Dr. John Opitz. We thank Ms. Jessica McVey for administrative support, and Dr. Jenni Tsafrir and Ms. Susan Knight for librarianship assistance. For the work cited here SRB was financially supported by the Institute for the Study of Man in Africa and the Kalahari Research Committee (South Africa); the Danish Research Foundation (Denmark); and the Medical Research Council and the Natural Sciences and Engineering Research Council (Canada). RPE is supported by the Holsclaw Family Professorship in Human Genetics and Inherited Diseases.

REFERENCES

Achermann JC, Ito M, Hindmarsh PC, Jameson JL. 1999. A mutation in the gene encoding steroidogenic factor-1 causes XY sex reversal and adrenal failure in humans. Nat Genet 22:125–126.

Alvesalo L, de la Chapelle A. 1981. Tooth sizes in two males with deletions of the long arm of the Y-chromosome. Ann Hum Genet 45:49–54.

Alvesalo L, Kari M. 1977. Sizes of deciduous teeth in 47, XYY males. Am J Hum Genet 27:486–489.

Ao A, Erickson RP, Winston RM, Handyside AH. 1994. Transcription of paternal Y-linked genes in the human zygote as early as the pronuclear stage. Zygote 2:281–287.

Arnold AP, Xu J, Grisham W, Chen X, Kim Y-W, Itoh Y. 2004. Minireview: Sex Chromosomes and brain sexual differentiation. Endocrinology 145:1057–1062.

Atkinson TG, Blecher SR. 1994. Aberrant anogenital distance in XX*Sxr* ("sex reversed") pseudomale mice. J Zool Lond 233:581–589.

Bagemihl B. 1999. Biological exuberance: Animal homosexuality and natural diversity. New York: St. Martins Press. 768 p.

Bardoni B, Zanaria E, Guioli S, Floridia G, Worley KC, Tonini G, Ferrante E, Chiumello G, McCabe ERB, Fraccaro M, Zuffardi O, Camerino G. 1994. A dosage sensitive locus at chromosome Xp21 is involved in male to female sex reversal. Nat Genet 7:497–501.

Baron D, Batista F, Chaffaux S, Cocquet J, Cotinot C, Cribil E, De Baeree E, Guiguen Y, Jaubert F, Pailhoux E, Pannetier M, Vaiman D, Vigier B, Veita R, Fellous M. 2005. *Fox12* gene and the development of the ovary: A story about goat, mouse, fish and woman. Reprod Nutr Dev 45:377–382.

Barrionuevo F, Bagheri-Fam S, Klattig J, Kist R, Taketo M, Englert C, Scherer G. 2006. Homozygous inactivation of *Sox-9* causes complete sex reversal in mice. Biol Reprod 74:195–201.

Bernstein R, Jenkins T, Dawson B, Wagner J, Dewald G, Koo GC, Wachtel SS. 1980. Female phenotype and multiple abnormalities in sibs with a Y chromosome and partial X chromosome duplication: H-Y antigen and Xg blood group findings. J Med Genet 17:291–300.

Birk OS, Casiano DE, Wassif CA, Cogliati T, Zhao L, Zhao Y, Grinberg A, Huang SP, Kreidberg JA, Parker KL, Porter FD, Wesphal H. 2000. The LIM homeobox gene *Lbx9* is essential for mouse gonad formation. Nature 403:909–913.

Bishop CE, Whitworth DJ, Qin Y, Agoulnik AI, Agoulnik IU, Harrison WR, Behringer RR, Overbeek PA. 2000. A transgenic insertion upstream of Sox-9 is associated with dominant XX sex reversal in the mouse. Nat Genet 26:490–494.

Blecher SR. 1972. Dermatoglyphics of the Pandamatenga Bush-Bantu hybrids. Hum Heredity 22:149–172.

Blecher SR. 1978. Microscopic epididymides in testicular feminisation. Nature 275:748–749.

Blecher SR. 1992. Amelogenin genes and sexual dimorphism of teeth in humans and mice. Am J Hum Genet 51:1451–1452.

Blecher SR, Erickson RP. 2007. Genetics of sex determination and differentiation. In: Polin RA, Fox WW, Abman SH, Eds. Fetal and neonatal physiology, 4th edition. Philadelphia: Saunders (in press).

Blecher SR, Wilkinson LJ. 1989. Non-Hormone-mediated sex chromosomal effects in development: Another look at the Y chromosome-testicular hormone paradigm. In: S.S. Wachtel, Editor. Evolutionary mechanisms in sex determination. Boca Raton, FL: CRC Press. p 219–229.

Blecher SR, Howie R, Li S, Detmar J, Blahut LM. 1999. A new approach to immunological sexing of sperm. Theriogenology 52:1309–1321.

Burgoyne PS. 1993. A Y-chromosomal effect on blastocyst cell number in mice. Development 117:341–345.

Camperio-Ciani A, Corna F, Capiluppi C. 2004. Evidence for maternally inherited factors favouring male homosexuality and promoting female fecundity. Proc R Soc Lond B 271:2217–2221.

Colvin JS, Green RP, Schmahl J, Capel B, Ornitz DM. 2001. Male-to-female sex reversal in mice lacking fibroblast growth factor 9. Cell 104:875–889.

Crisponi L, Deiana M, Loi A, Chiappe F, Uda M, Amati P, Bisceglia L, Zelante L, Nagaraja R, Porcu S, Ristaldi MS, Marzella R, Rocchi M, Nicolino M, Lienhardt-Roussie A, Nivelon A, Verloes A, Schlessinger D, Gasparani P, Bonneau D, Cao A, Pilia G. 2001. The putative forkhead transcription factor *FOX2* is mutated in blepharophimosis/ptosis/epicanthus inversus syndrome. Nat Genet 27:159–166.

Dart R. 1937. The physical characters of the *Auni-Khomani* Bushmen. Bantu Stud 11:175–246.

Epstein CJ, Smith S, Travis B. 1980. Expression of H-Y antigen on preimplantation mouse embryos. Tissue Antigens 15:63–67.

Erickson RP. 1999. Sex chromosome and autosome contributions to normal gonadal development. In: Pinsky L, Erickson RP, Schimle N. editors. Genetic disorders of human sexual development. New York: Oxford University Press. p 11–38.

Erickson RP, Stone JF, McNoe LA, Eccles MR. 1997. Molecular and clinical studies of three cases of female pseudohermaphroditism with caudal dysplasia suggest multiple etiologies. Clin Genet 51:331–337.

Evans HS, Buckton KE, Spowart G, Carothers AD. 1979. Heteromorphic X chromosomes in 46, XX males: Evidence for the involvement of X-Y interchange. Hum Genet 49: 11–31.

Fiddler M, Abdel-Rahman B, Rappdee DA, Pergament E. 1995. Expression of SRY transcripts in preimplantation human embryos. Am J Med Genet 55:80–84.

Ford CE, Jones KW, Polani PE, De Almeida JC, Briggs JH. 1959. A sex chromosome anomaly in a case of gonadal dysgenesis (Turner's syndrome). Lancet 1:711.

Garcia-Heras J, Corley N, Garcia MF, Kukolich MK, Smith KG, Day DW. 1999. De novo partial duplications 1p: Report of two new cases and review. Am J Med Genet 82:261–264.

Gardiner A, Brown MM, Gray JE. 1978. Unusual chromosomal variant in Klinefelter's syndrome. Br Med J 2:1123.

Garn SM, Lewis AB, Swindler DR, Kerewsky RS. 1967. Genetic control of sexual dimorphism in tooth size. J Dent Res 46:963–972.

Gibbons RJ, Picketts DJ, Villard L, Higgs DR. 1995. Mutations in a putative global transcriptional regulator cause X-linked mental retardation with alpha-thalassemia (ATR-X). Cell 80:837–845.

Gillman J. 1948. The development of the gonads in man, with a consideration of the role of fetal endocrines and the histogenesis of ovarian tumors. Contrib Embryol 32:81–131, (Carnegie Inst Pub No. 575).

Graves JAM. 1998. Interactions between *SRY* and *SOX* genes in mammalian sex determination. BioEssays 20:264–269.

Griffin JE, Wilson JD. 1980. The syndromes of androgen resistance. N Engl J Med 302:198–209.

Gubbay J, Collignon J, Koopman P, Capel B, Aconomou A, Munsterberg A, Vivian N, Goodfellow P, Lovell-Badge R. 1990. A gene mapping to the sex-determining region of the mouse Y chromosome is a member of a novel family of embryonically expressed genes. Nature 346:245–250.

Haraguchi R, Mo R, Hui CC, Makino S, Shiroishi T, Gaffield W, Yamada G. 2001. Unique functions of Sonic hedgehog signalling during external genitalia development. Development 128:4241–4250.

Heller NH, Blecher SR. 1982. Reverse, hormone-dependent sex difference in molar tooth mass in pubertal mice. Arch Oral Biol 27:325–329.

Holt SB. 1955. Genetics of dermal ridges. Frequency distributions of total finger ridge-count. Ann Hum Genet 20:159–170.

Huang B, Wang S, Ning Y, Lamb AN, Bartley J. 1999. Autosomal XX sex reversal caused by duplication of *SOX-9*. Am J Med Genet 87:349–353.

Imperato-McGinley J, Peterson RE, Gautier T, Sturla E. 1979. Androgens and the evolution of male-gender identity among male pseudohermaphrodites with 5α-reductase deficiency. New Eng J Med 300:1233–1237.

Jacobs PA, Strong JA. 1959. A case of human intersexuality having a possible XXY sex determining mechanism. Nature 183:302.

Jeays-Ward K, Dandonneau M, Swain A. 2004. *Wnt-4* is required for proper male as well as female sexual development. Dev Biol 276:431–440.

Jones KW, Singh L. 1981. Conserved repeated DNA sequences in vertebrate sex chromosomes. Hum Genet 58:46–53.

Jordan BK, Mohammed M, Ching ST, Delot E, Chen X-N, Dewing P, Swain A, Rao PN, Elejalde BR, Vilain E. 2001. Up-regulation of WNT-4 signalling and dosage-sensitive sex reversal in humans. Am J Hum Genet 68:1102–1109.

Josso N, Picard JY, Rey R, di Clemente N. 2006. Testicular anti-Mullerian hormone: History, genetics, regulation and clinical applications. Pediatr Endocrinol Rev 3:347–358.

Jost A, Vigier B, Prepin J, Perchellet JP. 1973. Studies on sex differentiation in mammals. Recent Prog Horm Res 29:1–41.

Just W, Rau W, Vogel W, Akherverdian M, Fredga K, Graves JA, Lyapunova E. 1995. Absence of *Sry* in species of the vole *Ellobius*. Nat Genet 11:117–118.

Katoh-Fukui Y, Tsuchiya R, Shiroishi T, Nakahar Y, Hashimoto N, Noguchi K, Higashinakagawa T. 1998. Male-to-female sex reversal in *M33* mutant mice. Nature 393:688–692.

Kiel-Metzger K, Warren G, Wilson GN, Erickson RP. 1985. Evidence that the human Y chromosome does not contain clustered DNA sequences (Bkm) associated with heterogametic sex determination in other vertebrates. N Engl J Med 313:242–245.

Kondo T, Zakany J, Innis JW, Duboule D. 1997. Of fingers, toes and penises. Nature 390:29.

Kondo M, Nanda I, Hornung U, Asakawa S, Shimizu N, Mitani H, Schmid M, Shima A, Schartl M. 2003. Absence of the candidate male sex-determining gene dmrt1(Y) of medaka from other fish species. Curr Biol 13:416–420.

Koopman P, Gubbay J, Vivian N, Goodfellow P, Lovell-Badge R. 1991. Male development of chromosomally female mice transgenic for Sry. Nature 351:117–121.

Lau EC, Mohandas TK, Shapiro LJ, Slavkin HC, Snead ML. 1989. Human and mouse amelogenin gene loci are on the sex chromosomes. Genomics 4:162–168.

LeBarr DK, Blecher SR. 1986. Epididymides of sex-reversed, XX mice lack the Initial Segment. Dev Genet 7:109–116.

LeBarr DK, Moger W, Blecher SR. 1991. Development of the normal XY and sex-reversed XXSxr pseudomale mouse epididymis. Mol Reprod Dev 28:9–17.

Levay SA. 1991. A difference in hypothalamic structure between heterosexual and homosexual men. Science 253:1034–1037.

Lubinsky MS. 1980. Female pseudohermaphroditism and associated anomalies. Am J Med Genet 6:123–136.

MacKenzie J, Chitayat D, McLorie G, Balfe W, Pandit PB, Blecher SR. 1994. Penoscrotal transposition: A Case Report and Review. Am J Med Genet 49:103–107.

McElreavy K, Vilain E, Abbas N, Herskowitz I, Fellous M. 1993. A regulatory cascade hypothesis for mammalian sex determination: *SRY* represses a negative regulator of male development. Proc Natl Acad Sci USA 90:3368–3372.

McLaren A, Simpson E, Tomonari K, Chandler P, Hogg H. 1984. Male sexual differentiation in mice lacking H-Y antigen. Nature 312:552–555.

Mittwoch U. 1993. Blastocysts prepare for the race to be male. Hum Reprod 8:1550–1555.

Mortlock DP, Innis JW. 1997. Mutation of *HOXA-13* in hand-foot-genital syndrome. Nat Genet 15:179–180.

Nef S, Verma-Kurvari S, Merenmies J, Vassalli JD, Efstratiadis A, Accili D, Parada LF. 2003. Testis determination requires insulin receptor family function in mice. Nature 426:291–295.

Nef S, Schaad O, Stallings NR, Cederroth CR, Pitetti JL, Schaer G, Malki S, Dubois-Dauphin M, Boizet-Bonhoure B, Descombes P, Parker KL, Vassalli JD. 2005. Gene expression during sex determination reveals a robust female genetic program at the onset of ovarian development. Dev Biol 287:361–377.

O W-S, Short RV, Renfree MB, Shaw G. 1988. Primary genetic control of somatic sexual differentiation in a mammal. Nature 331:716–717.

Ohno S, Smith JB. 1964. Role of fetal follicular cells in meiosis of mammalian oocytes. Cytogenetics 3:324–333.

Orkin CJ. 1970. The tablier in Griqua women. Leech 40:42–43.

Page DC, Mosher R, Simpson EM, Fisher EMC, Mardon G, Pollack J, McGillivray B, de la Chappele A, Brown LG. 1987. The sex-determining region of the human Y chromosome encodes a finger protein. Cell 51:1091–1104.

Pailhoux E, Vigier B, Chaffaux S, Servel N, Taourit S, Furet J-P, Fellous M, Grosclaude F, Cribiu EP, Cotinot C, Vaiman D. 2001. A 11.7-kb deletion triggers intersexuality and polledness in goats. Nat Genet 29:453–458.

Painter TS. 1923. Studies in mammalian spermatogenesis. II. The spermatogenesis of man. J Exp Zool 37:291–336.

Parma P, Radi O, Vidal V, Chaboissier MC, Dellambra E, Valentini S, Guerra L, Schedl A, Camerino G. 2006. R-spondin 1 is essential in sex determination, skin differentiation and malignancy. Nat Genet 38:1304–1309.

Payson RA, Wu J, Liu X, Chiu IM. 1996. The human FGF-8 gene localizes on chromosome 10q24 and is subjected to induction by androgen in breast cancer cells. Oncogene 13:47–53.

Peippo J, Bredbacka P. 1995. Sex-related growth rate differences in mouse preimplantation embryos in vivo and in vitro. Mol Reprod Dev 40:56–61.

Penrose LS. 1968. Medical significance of finger-prints and related phenomena. Br Med J 2:321–325.

Pergament E, Fiddler M, Cho N, Johnson D, Holmgren WJ. 1994. Sexual differentiation and preimplantation cell growth. Hum Reprod 9:1730–1732.

Perlof WH, Conger KB, Ley L. 1953. Female pseudohermaphroditism: A description of two unusual cases. J Clin Endocrinol 13:783–790.

Petit P, Moerman PH, Fryns JP. 1992. Full 69, XXY triploidy and sex-reversal: A further example of true hermaphroditism associated with multiple malformations. Clin Genet 41:175–177.

Polani PE, Polani N. 1979. Dermatoglyphics in the testicular feminization syndrome. Ann Hum Biol 6:417–430.

Ramsay M, Bernstein R, Zwane E, Page DC, Jenkins T. 1988. XX true hermaphroditism in southern African blacks: An enigma of primary sexual differentiation. Am J Hum Genet 43:4–13.

Raymond CS, Parker ED, Kettlewell JR, Brown LG, Page DC, Kusz K, Jaruzelska J, Reinberg Y, Flejter WL, Bardwell VJ, Hirsch B, Zarkower D. 1999. A region of human chromosome 9p required for testis development contains two genes related to known sexual regulators. Hum Mol Genet 8:989–996.

Salido EC, Yen PH, Koprivnikar K, Yu L-C, Shapiro LJ. 1992. The human enamel protein gene is expressed from both the X and the Y chromosomes. Am J Hum Genet 50:303–316.

Scherer G, Schempp W, Baccichetti C, Lensini E, Bricarelli FD, Carbone LD, Wolf U. 1989. Duplication of an Xp segment that includes the ZFX locus causes sex inversion in man. Hum Genet 81:291–294.

Scott WJ, Holson JF. 1977. Weight differences in rat embryos prior to sexual differentiation. J Embryol Exp Morphol 40:259–263.

Sekido R, Bar I, Narvaez V, Penny G, Lovell-Badge R. 2004. SOX-9 is up-regulated by the transient expression of SRY specifically in Sertoli cell precursors. Dev Biol 274:271–279.

Shan Z, Nanda I, Wang Y, Schmid M, Vortkamp A, Haaf T. 2000. Sex-specific expression of an evolutionarily conserved male regulatory gene, DMRT1, in birds. Cytogenet Cell Genet 89:252–257.

Shawlot W, Behringer RR. 1995. Requirement for *Lim 1* in head-organizer function. Nature 374:425–430.

Simpson E, Chandler P, Goulmy E, Disteche DM, Ferguson-Smith MA, Page DC. 1987. Separation of the genetic loci for the H-Y antigen and for testis determination on human Y chromosome. Nature 326:876–878.

Sinclair AH, Berta P, Palmer MS, Hawkins JR, Griffiths BL, Smith MJ, Foster JW, Frischauf A-M, Lovell-Badge R, Goodfellow PN. 1990. A gene from the human sex determining region encodes a protein with homology to a conserved DNA-binding motif. Nature 346:240–244.

Smith CA, McClive PJ, Western PS, Reed KJ, Sinclair AH. 1999. Evolution: Conservation of a sex-determining gene. Nature 402:601–602.

Sotou S, Mitsui Y, Tsuchiya K. 2001. Sex determination without the Y chromosome in two Japanese rodents Tokudaia osimensis osimensis and Tokudaia osimensis spp. Mamm Genome 12:17–21.

Swain A, Zanaria E, Hacker A, Lovel-Badge R, Camerino G. 1996. Mouse *Dax-1* expression is consistent with a role in sex

determination as well as in adrenal and hypothalamus function. Nat Genet 12:404–409.

Swain A, Narvaez V, Burgoyne P, Camerino G, Lovell-Badge R. 1998. *Dax-1* antagonizes *Sry* action in mammalian sex determination. Nature 391:761–767.

Tanaka Y, Okada Y, Hirokawa N. 2005. FGF-induced vesicular release of Sonic hedgehog and retinoic acid in leftward nodal flow is critical for left-right determination. Nature 435:155–166.

Thornhill AR, Burgoyne PS, 1993. A paternally imprinted X chromosome retards the development of the early mouse embryo. Development 118:171–174.

Tommmerup N, Schempp W, Meinecke P, Pedersen S, Bolund L, Brendt C, Goodpasture C, Guldberg P, Held KR, Reinwein H, Saugstad OD, Scherer G, Skjeldal O, Toder R, Westvik J, van der Hagen CB, Wolf U. 1993. Assignment of an autosomal sex reversal locus (*SRA1*) and campomelic dysplasia (*CMPD1*) to 17q24.3-q25.1. Nat Genet 4:170–174.

Vainio S, Heikkila M, Kispert A, Chin N, McMahon AP. 1999. Female development in mammals is regulated by Wnt-4. Nature 397:405–409.

Van Heyningen V, Hastie ND. 1992. Wilms' tumor: Reconciling genetics and biology. Trends Genet 8:16–21.

Vergnaud G, Page DC, Simmler MC, Brown L, Rouyer F, Noel B, Botstein D, de la Chapelle A, Weissenbach J. 1986. A deletion map of the human Y chromosome based on DNA hybridization. Am J Hum Genet 38:109–124.

Vidal VPI, Chaboissier M-C, de Rooij DG, Schedl A. 2001. *Sox-9* induces testis formation in XX transgenic mice. Nat Genet 28: 216–217.

Wachtel SS, Ohno S, Koo GC, Boyse EA. 1975. Possible role of H-Y antigen in primary sex determination. Nature 257:235–236.

Welshons WJ, Russell LB. 1959. The Y chromosome as the bearer of male determining factors in the mouse. Proc Natl Acad Sci USA 45:560–566.

Witschi E. 1948. Migration of the germ cells of human embryos from the yolk sac to the primitive gonadal fold. Contrib Embryol 32:67–80 (Carnegie Inst Pub No. 209).

Witschi E. 1956. Development of vertebrates. Philadelphia: W.B. Saunders Company. p 536–551.

Yadav BR, King WA, Betteridge KJ. 1993. Relationship between the completion of first cleavage and the chromosomal complement, sex, and developmental rates of bovine embryos generated in vitro. Mol Reprod Dev 36:434–439.

Zwingman T, Erickson RP, Boyer T, Ao A. 1993. Transcription of the sex determining region genes *Sry* and *Zfy* in the mouse preimplantation embryo. Proc Natl Acad Sci USA 90:814–817.