

Ovary? Testis? – A Mammalian Dilemma

Review

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Alfred Jost discovered that the presence of testes during mammalian embryogenesis results in male differentiation of the internal reproductive organs and external genitalia, while the presence of ovaries or the complete absence of gonads results in female differentiation (Jost, 1953). He deduced that the secretion of testicular hormones during a critical period in embryogenesis redirects differentiation of the reproductive tract from a female to a male pathway. Subsequently, the presence or absence of a Y chromosome was shown to correlate with male or female phenotype: XY and XXY individuals are males, while XO and XX individuals are females. Yet the studies by Jost made clear that the Y chromosome–encoded mediator of sex determination need only specify male gonadal development, and it was therefore called a testis-determining factor (designated *TDF* in humans and *Tdy* in mice).

It is now widely accepted that, apart from the gonads, all morphological differences between male and female eutherian mammals result from the action of two testicular hormones, Müllerian-inhibiting substance (MIS), also called anti-Müllerian hormone, and testosterone. MIS is secreted by Sertoli cells and causes regression of the Müllerian ducts, which would otherwise develop to form the uterus, cervix, fallopian tubes, and part of the vagina. Testosterone, secreted by Leydig cells, induces the development of male structures derived from the Wolffian duct, including the epididymis, vas deferens, and seminal vesicles. Formation of male rather than female external genitalia requires the conversion of testosterone into dihydrotestosterone by steroid 5 α -reductase in the target tissue. The embryogenesis of mammalian sexual dimorphism has thus been divided into primary and secondary sex differentiation, the former referring to the development of the bipotential gonads into testes or ovaries and the latter evocative of subsequent hormonal effects. This model may not apply to marsupials, in which the initial development of the scrotum, pouch, and mammary gland appears not to be controlled by gonadal hormones (O et al., 1988).

Here we will focus on the mechanism by which the sexual fate of the bipotential gonad is determined. This outcome is most directly assessed by histologic examination, as the ovary and testis have distinct tissue architectures. In this review, testicular histology will be equated with maleness, and ovarian histology will be equated with femaleness. Fertility does not figure into this definition of male or female, though individuals with disordered go-

nadal differentiation are most often sterile. Indeed, many individuals with disruptions of sex determination sought medical attention because of sterility and were then studied to localize the sex-determining function of the human Y chromosome (Page, 1986). A single gene, termed *SRY* (for sex-determining region Y) in humans and *Sry* in mice, has been identified by these efforts (Sinclair et al., 1990; Gubbay et al., 1990). While it is now clear that the presence or absence of *SRY* in the genome determines whether male or female gonadal differentiation takes place, many key questions remain to be addressed.

Of Mice and Men: The Case for *SRY*

In a remarkable set of experiments, Koopman et al. (1991) demonstrated that XX mice differing from their sisters by the addition of a single DNA fragment, 14 kb of Y chromosome including the *Sry* gene, can develop as phenotypic males. Though devoid of spermatogonia, the testes of these transgenic XX male mice appear to have normal hormonal function, and the mice display normal male secondary sexual differentiation and mating behavior. In contrast, XY mice differing from normal XY males only by the deletion of a Y DNA fragment, 11 kb including the *Sry* gene, develop as phenotypic females. These XY female mice are fertile and can transmit the mutant Y chromosome to their offspring (Lovell-Badge and Robertson, 1990; Gubbay et al., 1992). Thus, as judged by gonadal histology and hormone production, the presence or absence of *Sry* is sex determining in mice.

Interpretation of the data from humans is less straightforward, though rare “sex-reversed” individuals provide counterparts to both XX male and XY female mice. Crossing over normally occurs near the short arm telomeres of the human X and Y chromosomes during male meiosis, and aberrant recombination occurring more proximally can transfer fragments of Y-specific DNA (including *SRY*) into the genome of an otherwise XX individual. Most such translocations are cytologically undetectable, and the resulting XX males do not produce spermatozoa but have normal male internal and external genitalia. Thus, in most instances, the phenotype of the XX male transgenic mice is duplicated in humans. However, when especially small (35 kb) fragments of Y-specific DNA are translocated, the resulting XX+*SRY* individuals invariably have genital abnormalities, sometimes despite apparently normal testicular histology (Palmer et al., 1989). These abnormalities may include “sexually ambiguous” external genitalia (incomplete fusion of the labioscrotal folds with a resultant bifid scrotum or displaced urethral opening), undescended testes, or ovotestes (gonads containing areas of both testicular and ovarian histology) with persistence of female structures derived from the Müllerian duct.

There are several possible explanations for the phenotypic differences between these XX+*SRY* humans and XX+*Sry* transgenic male mice. Perhaps the most likely scenario is that *SRY* transcription is reduced in XX+*SRY* humans (Palmer et al., 1989). This could result from the

spread of X inactivation across a nearby X;Y translocation breakpoint in humans. The murine *Sry* transgene would not be subject to this effect if it inserted at an autosomal locus. Alternatively, loss of regulatory sequences 5' of the human *SRY* gene might result from X;Y translocations involving only small amounts of Y-specific DNA. It is unclear whether reduced expression of *SRY* could explain the phenotype; presumably decreased or delayed hormonal action would have to result. It remains a formal possibility that, in humans (but not mice), full development of the male phenotype requires expression of a second Y-linked gene, located near *SRY* and perhaps expressed outside the testis.

In contrast with XY female mice, XY female humans are sterile. Some of these individuals have mutations affecting the *SRY* open reading frame (Berta et al., 1990; Jäger et al., 1990). Such XY individuals fail to develop mature testes or ovaries and instead form poorly differentiated gonads without clear male or female histology. Because the gonads lack male hormonal function, Müllerian structures persist and female secondary sexual differentiation follows. Infertility may result in part from X chromosome monosomy: unlike XO mice, XO humans are sterile.

SRY itself encodes a member of the high mobility group (HMG) family of DNA-binding proteins. Like other members of this family, *SRY* protein has been shown to bind to the minor groove of the double helix in a sequence-specific manner and is predicted to bend the target DNA by at least 85° and perhaps as much as 130° (Nasrin et al., 1991; Harley et al., 1992; Giese et al., 1992; van de Wetering and Clevers, 1992). Analysis of the predicted amino acid sequence reveals no obvious activation domain, and only the HMG (DNA-binding) domain is conserved among mammals (Foster et al., 1992). Moreover, none of the *SRY* point mutations reported in human XY females is outside the HMG domain. Thus, it is possible that DNA bending is the sole mechanism by which *SRY* acts in sex determination. By bringing together distant DNA sequences or altering chromatin structure, *SRY*-induced bending might activate or repress target gene expression.

When and Where Does Sex Happen?

The mammalian gonad is composed of germ cells and three types of somatic cells. Primordial germ cells, which are first observed in the extraembryonic mesoderm (Ginsburg et al., 1990), migrate into the primitive gonad at 10.5–12 days post coitum (dpc) in the mouse. The somatic portion of the gonad is composed of supporting cells (Sertoli cells in the male and follicle cells in the female, thought to derive from common progenitors), steroidogenic cells (Leydig cells in the male and theca cells in the female), and connective tissue cells.

When is gonadal sex determined? Though the sexual fate of an embryo is set at fertilization, it is not known when gonadal cell lineages become committed to male or female development. XX and XY mouse embryos are morphologically indistinguishable until 11.5–12.5 dpc, when pre-Sertoli cells align to form testis cords in the XY gonad. By this time a male or female developmental program clearly has been initiated. Yet it is not obvious that sex determi-

nation (commitment) is coincident with the onset of sex differentiation (e.g., histologic change). When does the presence or absence of *Sry* protein result in irreversible commitment to male or female differentiation? Barring pleiotropic effects of *Sry*, it is reasonable to suppose that sex determination begins when *Sry* is first expressed. Initial experiments suggested that this is from 10.5 to 12.5 dpc in the murine gonad (Koopman et al., 1990). It remained possible that *Sry* expression began still earlier and that transcripts were not detected because RNA from earlier stages was extracted from pooled whole embryos rather than from isolated gonads. Indeed, *Sry* expression in the preimplantation mouse embryo has been reported (Zwingman et al., 1993). Whenever the onset of *Sry* transcription, it is difficult to judge the point at which cells become committed to male or female differentiation in the absence of conditional mutants. If only one could fully exploit temperature-sensitive alleles in mammals!

If it is unclear when sex determination begins, it is equally unclear when it finishes. By 13.5 dpc, *Sry* transcripts are no longer detectable by polymerase chain reaction amplification of gonadal RNA (Koopman et al., 1990). While it is not known how long *Sry* protein persists, the function of the Y chromosome in determining the sex of the embryo appears to be complete by this time. Given that other proteins must be involved, sexual commitments could still be in flux at the time of the disappearance of *Sry*.

In which cells do the initial steps in gonadal sex determination occur? Not in the germ cells: in mouse embryos that are defective in migration of primordial germ cells to the gonad (because of mutations at the *W* or *Sf* loci), the process of gonadal differentiation is otherwise undisturbed (reviewed by McLaren, 1991). XY animals still develop testes, and XX animals still develop ovaries. Sex determination must unfold in the somatic cell lineages of the gonad.

In which of the somatic lineages, then, does gonadal sex determination occur? Experiments involving XX↔XY chimeric mice, reminiscent of mosaic analysis in invertebrates, have sought to identify gonadal cell lineages that become committed to male or female development as a direct result of the presence or absence of the Y chromosome. These should be the cell lineages in which presence or absence of *Sry* is pivotal and in which sex determination is initiated. If sex determination occurs autonomously in each gonadal cell, then all of the XY cells in a chimeric gonad should be male and all of the XX cells should be female. If, on the other hand, the sexual phenotype of a gonadal cell is determined in consultation with its neighbors, then the strict correlation of chromosome constitution with cellular phenotype should break down.

In chimeric mice with testes, the proportions of XX and XY cells in the Leydig population are similar to those seen outside the gonad, implying that sex determination does not occur autonomously in Leydig cell precursors. In contrast, most but not all Sertoli cells are XY, indicating some direct action by the Y chromosome, presumably by *Sry* (Palmer and Burgoyne, 1991a; Patek et al., 1991). The presence of a few XX Sertoli cells in XX↔XY embryos

indicates that cells lacking *Sry* can be recruited into the Sertoli population. The mechanism by which such recruitment occurs is unknown. Complementary studies in XX↔XY chimeras with ovaries show that XY precursors can become follicle cells, the female counterparts of Sertoli cells.

Two major conclusions can be drawn from these experiments. First, the initial steps that commit the gonad to male or female differentiation likely occur in the supporting cell lineage (i.e., in pre-Sertoli or prefollicle cells). Since *Sry* encodes a nuclear factor and must be expressed and function within the cells that carry out these steps, it is presumably expressed in pre-Sertoli cells. This prediction has yet to be confirmed or refuted. Second, cell-autonomous action of *Sry* is neither necessary nor sufficient to cause histologic Sertoli cell differentiation; intercellular interactions are required. Indeed, there may be no sexually dimorphic cell fates that are autonomously determined in mammals, not even in the gonad.

Autosomes and Sex Determination:

The Untold Story

Sex chromosomes specify male or female phenotype in both the fruitfly *Drosophila melanogaster* and the nematode *Caenorhabditis elegans*, yet most of the genes involved in sex determination in these organisms are located on autosomes (reviewed by Hodgkin, 1990). The same is likely to be true of mammals, and this supposition is supported by the "unexplained" majority of human XY females, in whom *SRY* appears to be intact and in whom one might suspect mutations in autosomal or X-linked sex-determining genes. A minority of human XX males appear to carry no portion of the Y chromosome, not even *SRY*, and here too one might suspect autosomal or X-linked mutations. One can envision a pathway for sex determination in which *SRY* is the only Y-linked factor. Autosomal or X-linked genes participating in the pathway could act upstream or downstream of *SRY*.

A purely genetic approach to identifying autosomal genes involved in murine sex determination is based on the observation that when the *Mus poschiavinus* Y chromosome (Y^{POS}) is placed on the C57BL/6 genetic background by breeding, XY^{POS} females and XY^{POS} hermaphrodites result (Eicher and Washburn, 1986). These females and hermaphrodites have acquired a C57BL/6 autosomal allele(s) that suppresses the masculinizing effect of the POS *Sry* allele but does not suppress the masculinizing effect of the C57BL/6 *Sry* allele. The complex pattern of inheritance of this XY sex reversal suggests that more than one autosomal gene is involved. If the genetic background effect is due to a small number of genes, then it should be possible to map those genes by linkage analysis.

One advantage of such a genetic approach is that it does not require a priori assumptions as to whether the products of these autosomal genes act upstream or downstream of *Sry*. The functionally important differences between the C57BL/6 and *M. poschiavinus* *Sry* alleles may be in the regulatory sequences or in the encoded protein; the existing genetic and molecular findings do not reveal which is the case. By histologic criteria, testis differentia-

tion in the presence of Y^{POS} occurs later during embryogenesis than in the presence of $Y^{C57BL/6}$ (Palmer and Burgoyne, 1991b). Eicher and Washburn (1986) have suggested that the Y^{POS} testis-determining signal fails to preempt the C57BL/6 program for ovarian differentiation.

Target genes immediately downstream of *SRY* have not yet been identified, though *MIS* would seem to be a candidate. First, the work by Jost (1953) demonstrated that *MIS* functions subsequent to the testis-determining factor. Second, apart from *SRY*, *MIS* is the first biochemical marker of male differentiation. The *MIS* gene is autosomal in both humans and mice (King et al., 1991). In situ hybridization experiments in mice first detect *Mis* expression at 12.5 dpc in Sertoli cells, approximately 48 hr after the onset of *Sry* transcription (Münsterberg and Lovell-Badge, 1991). This observation is consistent with the possibility that *Sry* protein directly activates *Mis*, yet the delayed onset of *Mis* expression leaves open the possibility that other steps are interposed.

Even though *MIS* acts downstream of *SRY* in sex differentiation, it is not obvious that *MIS* functions in gonadal sex determination strictly defined. *MIS* can influence gonadal development: ovaries exposed to exogenous *MIS* lose oocytes and develop cord-like structures reminiscent of testes. This so-called freemartin phenomenon was first noted in female calf fetuses sharing placental circulation with a male twin and has been simulated in organ culture and in XX mice transgenic for human *MIS* (Vigier et al., 1989; Behringer et al., 1990). However, exposure of the XX embryonic gonad to *MIS* does not result in true testicular histology. Targeted disruption of the *Mis* gene in XY mice has not yet been reported, though three XY human siblings homozygous for a nonsense mutation in *MIS* have been identified (Knebelmann et al., 1991). Each of these brothers has a uterus, fallopian tubes, and undescended testes with apparently normal histology, arguing that *MIS* does not function in testis determination.

Given the common embryologic origin of the kidney and gonad, it is not surprising that *WT1*, a gene first identified for its role in childhood kidney cancer (Wilms' tumor), also appears to participate in gonadal development (reviewed by Hastie, 1992). The evidence is of three sorts. First, XY humans who are heterozygous for certain mutations in *WT1* (Denys-Drash syndrome) share several phenotypic features with XY humans mutant in *SRY*: disordered gonadal histology, persistent Müllerian derivatives, and partial or complete feminization of the external genitalia (Bruening et al., 1992; Pelletier et al., 1991a; Baird et al., 1992). Second, the predominant sites of fetal *WT1* expression are tissues that develop into kidney and gonad, and transcription is first detected at 9 dpc (Pelletier et al., 1991b; Armstrong et al., 1992). Third, targeted disruption of the murine *WT1* gene largely prevents thickening of the intermediate mesoderm to form undifferentiated gonadal tissue (Kreidberg et al., 1993). This arrest of gonadal development was noted in all fetuses homozygous for the *WT1* null allele. Though the identity of the sex chromosomes was not determined in each case, presumably both XX and XY embryos were included in the analysis. The gonad is histologically neither male nor female at this stage and is

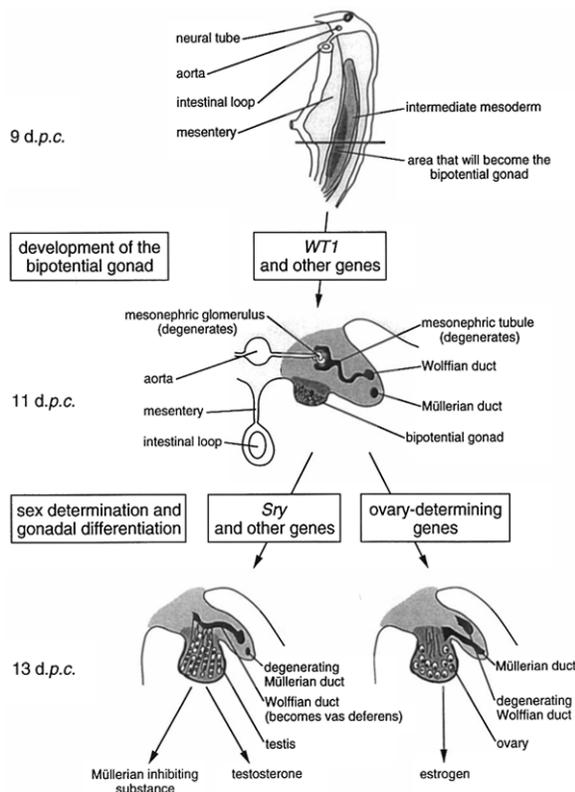


Figure 1. Two Stages in Gonadal Differentiation

First, a portion of the intermediate mesoderm thickens to give rise to the bipotential gonad. Second, the bipotential gonad differentiates into either a testis or an ovary. *WT1* likely participates in the first stage and may participate in the second stage as well. *Sry* does not function in the generation of the bipotential gonad, but directs subsequent gonadal differentiation. Approximate times during murine development are shown at left. It is possible that sex determination initiates earlier than is indicated here. The horizontal line across the top diagram indicates the level of the transverse sections depicted in the subsequent diagrams.

thought to be bipotential. This mutant phenotype suggests that *WT1*, a zinc finger transcription factor, acts well upstream of *SRY* and of sex determination and that its targets include genes other than *SRY* (Figure 1). It is possible that *WT1* also acts directly on *SRY*. However, if *SRY* were the only gonadal target, disruption of *WT1* should have resulted in formation of normal ovaries in both XX and XY embryos. The case of *WT1* highlights the difficulty and importance of determining whether a gene participates directly in sex determination or in the more upstream process of forming the bipotential gonad. Careful histologic analysis of both XX and XY gonads will be important in interpreting mutations in autosomal or X-linked genes postulated to function in gonadal sex determination.

There is no evidence that *SRY* functions in developmental pathways other than gonadal sex determination, yet this need not be the case for other sex-determining genes. In *Drosophila*, the transcription factor encoded by the *daughterless* gene functions in both sex determination and neural development. In humans, a possible example of such pleiotropy is provided by campomelic dysplasia, an autosomal recessive syndrome of severe skeletal ab-

normalities (in both XX and XY individuals) and varying degrees of sex reversal (in XY individuals only). It is possible that these abnormalities result from mutation in a single protein that functions in both skeletal and gonadal development, though it may also be that two nearby genes are disrupted in individuals with this syndrome. Chromosomal anomalies in a few affected individuals suggest that the campomelic dysplasia gene is located on the distal long arm of chromosome 17 (Tommerup et al., 1993). The gonadal phenotype in campomelic dysplasia could result from mutation of a gene involved in forming the bipotential gonad (like *WT1*) or involved in gonadal sex determination strictly defined (like *SRY*; Figure 1).

At the molecular level, the process that determines the fate of the bipotential gonad is largely unexplored both upstream and downstream of the function of *SRY*. Biochemical and genetic studies may soon reveal other sex-determining factors. Upstream of *SRY*, there must exist an upstream transcriptional milieu in the mammalian bipotential gonad such that *SRY*, if present in the genome, will be expressed. This milieu may be specific to undifferentiated gonadal supporting cells, and it could include the presence of *WT1* and other transcription factors encoded by autosomes or the X chromosome. A functional parallel in *Drosophila* sex determination is provided by the *daughterless* gene product, which is required to initiate embryonic expression of *Sex-lethal*, the master gene controlling *Drosophila* sex phenotype (Cline, 1993). Downstream of *SRY*, individual gonadal cells do not decide their sexual fates autonomously, and the critical cell interactions are likely mediated by autosome or X-encoded proteins. These proteins have not yet been identified. *MIS* is one candidate, though its functions may be entirely subsequent to gonadal sex determination. Other proteins participating in such sex-determining cell interactions may be uncovered by studies of unexplained human XY females and XX males and by analysis of genetic background effects causing sex reversal in mice.

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