Genetics of Mammalian Sex Determination: Some Unloved Exceptions

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ABSTRACT
The genetics of sex determination is a child of the twentieth century, which overturned the previously held view that sex was determined by the environment. The last quarter of the century witnessed an active search for sex-determining genes in mammals. Although successful, the modus operandi of these genes remained unknown, and the relationship between the sex-determining systems of mammals and other vertebrates remained enigmatic. To overcome these problems, scientists in the 21st century should heed William Bateson's counsel to treasure exceptions, for they point the way to progress. One exception to conventional concepts of sex determination is the bilaterally asymmetrical distribution of ovaries and testes in true hermaphroditism. Ovaries favour the left side in humans and the right side in mice. Observations suggesting that a reversal of asymmetry may occur with increasing organ size may point to a possible explanation. A reevaluation is also required regarding the beginning of sex differentiation, in view of mounting evidence of a sex difference in growth rates of early embryos. Another question to be settled is whether the function of SRY is confined to the fetal gonad. The recent demonstration that Sry induces cell proliferation in the fetal mouse gonad (Schmahl et al., 2000) further emphasizes the importance of differential growth in sex determination and differentiation. It is suggested that SRY represents an additional growth-promoting gene sequestered by mammals to enable the XY embryo to undergo male sex differentiation in the female hormonal environment of the uterus. An increased awareness of the relationship between growth and gonadal differentiation should lead to a better understanding of sex determination in mammals and an ability to relate the function of sex-determining genes to the effects of environmental factors. J. Exp. Zool. 290:484–489, 2001. © 2001 Wiley-Liss, Inc.

LAYING THE FOUNDATIONS
The genetics of sex determination is a child of the twentieth century, which overturned the previously held view that sex was determined by environmental conditions (Mittwoch, '73, '85). The century began with the rediscovery of Mendel's laws by three independent European investigators (Correns, 1900; de Vries, 1900; Tschermak, 1900). In the years that followed, scientists in the USA discovered sex chromosomes in insects. An earlier finding by Henking (1891) in Göttingen, Germany, had established that as a result of meiosis in the plant bug, Pyrrhocoris apterus, two kinds of spermatocytes were formed, one of which contained a large chromatin element, labeled X, which was absent in the other. This led McClung (1901), of Kansas University, to suggest that the odd chromosome was connected with the determination of sex (Mittwoch, '73, '83).

Although the idea that sex was determined by a chromosome rather than by external conditions met with much scepticism, two investigators obtained evidence in favour of the chromosome theory. E.B. Wilson ('05) studied chromosomes in insects and found two different dimorphisms in spermatocytes: either a chromosome was present in one class and absent in another, or both classes possessed a chromosome of different size. Netty Stevens ('05) investigated both sexes of the common mealworm, Tenebrio molitor, and found that in males, but not in females, one chromosome was smaller than the others, and concluded that this chromosome must be responsible for the production of males. The smaller chromosome became known as the “Y chromosome,” its partner as the “X chromosome” (Wilson, '09), and both as “sex chromosomes” (Wilson, '11).

By the end of the first quarter century it was known that the XXXY mechanism of sex deter-
mination (or its variant XX/XO) occurred in many species of insects, including *Drosophila melanogaster*, as well as in several species of dioecious flowering plants, whereas birds and Lepidoptera had the apparently opposite ZZ/ZW mechanism (Morgan, ’28).

As regards man and other mammals, the pattern of sex-linked inheritance indicated that the mechanism had to be either XX/XY or XX/XO (Morgan, ’28). During the course of the second quarter century, evidence in favour of the existence of a Y chromosome increased (Matthey, ’49), but conclusive evidence had to await the development of new cytogenetic techniques in the third quarter of the century.

**CYTOGENETICS AND MOLECULAR GENETICS**

In addition to revealing the normal male and female karyotypes, the new cytogenetic techniques uncovered a number of sex chromosomal abnormalities which demonstrated that the mammalian Y chromosome plays a dominant role in determining the development of the male phenotype. Thus the function of the mammalian Y chromosome was found to differ from that of *D. melanogaster*, in which XXY individuals are female, and to resemble that of the red campion, *Silene* (formerly *Melandrium* dioica, in which plants with the XXXY constitution are male (Warmke, ’46).

Based on the premise that the fetal mammalian testis plays an essential role in the development of the male phenotype (Jost et al., ’73), the last quarter of the twentieth century was characterized by the search for the testis-determining factor, *TDF*. This culminated in the isolation of the *SRY* gene and its mouse homologue, *Sry*; the latter was found to be capable of effecting testicular differentiation in transgenic XX mice (Koopman et al., ’91). It became apparent, however, that the function of the gene was dependent on other, non-Y-chromosomal, genes, and by the end of the century, scientists had accumulated an impressive array of candidate genes implicated in sex determination (Swain and Lovell-Badge, ’99). Nevertheless, the modus operandi of sex-determining genes in the development of individuals remained unknown. Equally puzzling, in view of the centrality of *SRY* in mammalian sex determination and its accepted role as testis-determining factor, was the realization that a sex-specific *SRY* gene is absent in nonmammalian vertebrates (Pask and Graves, ’99).

In trying to solve these problems, the geneticists of the 21st century might do well to hark back to a pioneer of the twentieth, the British biologist William Bateson, to whom we owe the term genetics (Bateson, ’28).

**TREASURE YOUR EXCEPTIONS**

Bateson first used the word genetics in a letter in 1906. Two years later (Bateson, ’08), he gave an inaugural lecture entitled “The Methods and Scope of Genetics,” which includes this admonition to the students of Cambridge University: “Treasure your exceptions! . . . Keep them always uncovered and in sight. Exceptions are like the rough brickwork of a growing building which tells us that there is more to come and shews where the next construction is to be.”

Judging by the literature on sex determination, Bateson’s counsel is, in the words of Hamlet, “more honor’d in the breach than the observance.” To redress the balance, I will briefly mention a few data that are often omitted.

**BILATERAL ASYMMETRY IN TRUE HERMAPHRODITISM**

One of the exceptions that proves the rule of the genetics of sex determination is the existence of both ovarian and testicular tissue in individuals with but a single cell line. With the exception of a small minority of chimeric patients with cell lines of more than one genetic constitution, true hermaphroditism requires an explanation of either the presence of ovarian tissue in the presence of *SRY*, or the presence of testicular tissue in the absence of the gene. A further complication is added by the fact that the two types of tissues are not symmetrically distributed within the body. When large series of patients are examined, it is found that ovaries occur about twice as often on the left as on the right side, whereas testes and ovotestes occur more often on the right (Fig.1) (van Niekirk and Retief, ’81; Krob et al., ’94).

The asymmetrical differentiation of gonads in human true hermaphroditism is reminiscent of the situation in birds (Lillie, ’52) and should serve as a reminder that, even in mammals, there is an epigenetic contribution to sex differentiation. This is likely to be based on a difference in growth rate between left and right fetal gonads. A comparison of human fetal gonads has shown that those on the right are on average more developed than those on the left (Mittwoch and Mahadevaiah, ’80), suggesting that left gonads may have an inherently greater chance of becoming ovaries,
whereas right goads are a little more likely to develop testicular tissue.

Hermaphrodite mice also exhibit bilateral asymmetry, but in the opposite direction: testes are more frequently situated in the left and ovaries on the right side (Eicher and Washburn, '83; Ward et al., '87; Biddle et al., '91, '93). The total number of gonads in unilateral and bilateral hermaphrodite mice listed in these four publications is as follows: ovaries, 30 on the left and 61 on the right; testes, 212 on the left and 36 on the right; ovotestes, 80 on the left and 225 on the right. Most ovotestes were partnered by a left testis.

The difference in the direction of asymmetry between humans and mice could be connected with the difference in size between human and murine fetal gonads. An analysis of kidney weights in wild mice showed that right kidneys tended to be heavier than those on the left, but suggested that this effect decreased with increasing kidney weight and that the direction of asymmetry may eventually be reversed (Mittwoch, '79). In contrast to mice, Henry Gray (1858) wrote of human kidneys that "the left is nearly always heavier than the right, by about two drachms."

A relationship between bilateral asymmetry and organ size is further substantiated by an analysis of left and right gonadal volumes in embryos of the grey short-tailed opossum, *Monodelphis domestica*, in which gonad volumes averaged less than 0.01 mm³, and which showed a highly significant difference in favour of the left gonad (Baker, '93). In newborn opossums, this difference was reduced and no longer significant.

**DOES SEX DIFFERENTIATION BEGIN AT CONCEPTION?**

Based on morphological criteria, the conventional view has been that the gonad develops in a non-sex-specific manner, until the action of *SRY* triggers the differentiation of the Sertoli cell lineage, which in turn directs the differentiation of the other cell of the testis (Swain and Lovell-Badge, '99). This view, however, fails to consider quantitative data suggesting that accelerated growth is the first phenotypic characteristic of developing testes (Mittwoch, '86). Moreover, side by side with the search for genes involved in testis differentiation, which characterized the last decades of the past century, an impressive, though less publicized, body of data accumulated that cast doubt on the idea that the switch directing the indifferent gonad into the testicular pathway is the first step of sexual differentiation.

Scott and Holson ('77) found that in 12-day-old rat embryos, males were heavier than females, and their protein content was higher. Since the gonads were not yet sexually differentiated, the authors postulated the existence of sex-linked genes influencing body growth prior to gonadal endocrine activity. A similar conclusion was arrived at by Pedersen ('80) on the basis of a longitudinal study by ultrasound of human fetuses. It showed that at 8 to 12 menstrual weeks, female fetuses were one day behind male fetuses in crown-rump length and biparietal diameter of the skull, a difference that rose to between six and seven days at term. Extrapolation of the curve suggested that it would intercept the time axis a few weeks after conception, suggesting that at least part of the difference in growth rates between males and females is encoded at conception and determined by the sex chromosomes.
A sex difference in the developmental rate of early embryos was established experimentally by Tsunoda et al. ('85). Mouse blastocysts were divided into three classes—fast, intermediate, and slow, according to the time of blastocoel formation, before being implanted into foster mothers. It transpired that most fast embryos developed into males and the majority of slow embryos into females, while the sexes of the intermediate embryos occurred in roughly equal numbers. An essentially similar difference in development of male and female bovine blastocysts produced in vitro was found by Avery et al. ('91) and by Xu et al. ('92), demonstrating that the cause is developmental rate of the embryo, rather than time of fertilization.

In human embryos produced in vitro, male embryos had on average more cells than female embryos on day 2 after insemination, and there was evidence of increased metabolic activity in male embryos between days 2 and 5 (Ray et al., '95). Evidence of increased metabolic activity had previously been reported in male preimplantation bovine embryos (Tiffin et al., '91).

Zwingman et al. ('93) reported that both the Sry and the Zfy genes of mouse embryos are already expressed in two-cell mouse embryos, and Ao et al. ('94) found Y-linked genes to be expressed in the pronuclear stage of human zygotes. As yet, no consensus has been reached regarding the early expression of sex-determining genes (Edwards and Beard, 2000). There is increasing evidence, both in humans and in reptiles, that differences in growth rates at early stages in development can exert profound effects in later life. Differences in fetal growth parameters have been implicated in differences in blood pressure in 50-year-old men (Leon et al., '96). In reptiles, temperature is an important factor in influencing developmental rates, which, in addition to sex, can affect vertebrae numbers and some morphometric characteristics, as well as other variables affecting lifetime fitness (Merchant-Larios et al., '97; Braña and Ji, 2000). The assumption that pregonadal growth differences are unlikely to affect the process of gonadal sex differentiation is in need of revision.

**THE FUNCTION OF SRY**

The original hypothesis of a causal link between sex differentiation and growth (Mittwoch, '69) has received strong support from the recent report by Schmahl et al. (2000) that Sry induces cell proliferation in the rudimentary gonad of mouse embryos, and that this was the earliest detected effect of Sry expression in the system under investigation. This finding raises the question as to the function of other sex-determining genes, and also whether SRY functions exclusively in the differentiating testis. Expression of SRY has been reported in many tissues of human fetuses, including heart, liver, and kidney (Clépet et al., '93). A direct, although perhaps subtle, effect of SRY on the growth of these organs remains a possibility.

The demonstration that Sry acts by accelerating the rate of growth in the developing gonad helps to solve the puzzle of why the “testis-determining” gene is present only in mammals. It can be simply explained as an adaptation to the reproductive biology of eutherian mammals, in which development of both sexes occurs within the female hormonal environment of the uterus (Short, '74; Mittwoch, '93; Wolf, '99). In this new hormonal environment, the role of oestrogen as an agent of female sex differentiation had to be abandoned in favour of testosterone, which is required at an early stage by the developing male. We may surmise that during the evolution of mammals most species sequestered an additional growth-promoting gene to enable the XY embryo to win the race to be male.

The scientists of the 21st century are now in a position to construct the genetics of sex determination into a coherent system and to relate the function of the sex-determining gene SRY to that of environmental factors, particularly temperature, which it replaced.

**NOTE ADDED IN PROOF**

Since this paper was submitted, two publications have appeared that provide further evidence on the relationship between growth and sex differentiation. Colvin et al. (2001) reported that most XY mice lacking fibroblast growth factor 9 developed as females. Conversely, XX mice carrying a Sox9 transgene were found to develop as males (Vidal et al., 2001). The fact that Sox9 induces testicular differentiation even in the absence of Sry suggests the likelihood that Sox9 also increases cell proliferation.

**LITERATURE CITED**
