THE MOLECULAR PURSUIT OF MASCULINITY

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ABSTRACT

Ever since Aristotle suggested that females are incomplete males, the study of sex determination has been a persistent reiteration of this notion. Thus, the scientific study of sex determination has centred male specific single factors to explain this phenomenon. These single factors include testes, testosterone, the Y chromosome, H-Y antigen and, finally, a recently cloned gene called ZFY. It is evident that such an androcentric bias is incapable of explaining sex determination.

RÉSUMÉ

Depuis qu' Aristotle à suggéré que les femelles sont des mâles incomplets, l'étude de la détermination du sexe persiste à réiterer cette idée. Les études scientifiques de la détermination du sexe se sont concentrées sur des facteurs singuliers spécifiquement mâles afin d'expliquer ce phénomène. Les facteurs singuliers incluent les testicules, le testosterone, le chromosome Y, l'antigène H-Y et, enfin, un gène récemment cloné nommé ZFY. Il est evident qu'un biais androcentrique est incapable d'expliquer la détermination du sexe.

INTRODUCTION

This paper will discuss the historical context of research on sex determination and differentiation of the female and male during embryonic development. A description of embryonic development, a discussion of the role of the mammalian Y chromosome, and a review of a recently cloned gene that may be responsible for sexual determination serves to demonstrate the androcentric paradigm within which this research has taken place. Indeed, the search for the mechanism of sex determination is perhaps more aptly described as the molecular pursuit of masculinity.

THE DEVELOPMENT OF FEMALES AND MALES: AN HISTORICAL PERSPECTIVE

Androcentric ideas about the way the sex of an infant is determined have been put forward at least since the time of the early Greeks. For Aristotle, the female was

> ... an impotent male, for it is through a certain incapacity that the female is female, being incapable of concocting the nutriment in its last stage into semen ... (Agonito 1977:44)

The persistence of this notion can be seen in the writings of the philosopher David Hume who suggests in *A Treatise of Human Nature* that "the principle of generation goes from the man to the woman" (Agonito 1977:125). The idea of woman as an incomplete male has been strengthened in the twentieth century. Freud's introduction of the concept of penis envy, for example, suggested that woman was only a castrated male (Agonito 1977:297). Indeed, there is little doubt that biological and social scientific research was used to assert male superiority (Martin 1987:32).

The current framework within which sex determination is studied (developed in the late forties to early fifties) reflects this intellectual legacy. By castrating rabbit embryos, Alfred Jost was able to show that the fetal rabbit acquired a female phenotype, as defined by its secondary sexual characteristics (Wilson, George and Griffin 1981:1278). Removal of the undifferentiated gonads, moreover, prompted the developing embryo to differentiate into a female phenotype (Wilson, George and Griffin 1981:1278). These experiments were interpreted to mean that the male phenotype is defined by the presence of testes, while the female phenotype will occur without gonads. Since the secondary female phenotype resulted from the removal altogether of the gonads, it appeared that sex determination could be reduced to testicular determination (McLaren 1988:4; Jost and Magre 1988:56; Burgoyne 1988:64). Females were castrated males, it seemed.

Several important consequences of Jost's experiments are evident in sex determination research today. First, the primary sex characteristics (the gonads) of males and females are considered to give rise to the secondary sexual characteristics (Haseltine and Ohno 1981:1274). Second, researchers still fail to take into account that development of the secondary sexual phenotype in females is not solely governed by the

presence of ovaries. Third, although Jost conducted his experiments forty years ago, some researchers are still mesmerized by their implications, despite the fact that the results were inconclusive. Indeed, very few of his experimental animals actually survived the surgical molestation (Wilson 1989:324). The experiments, moreover, were never replicated, despite several attempts by other researchers (Wilson 1989:324). In spite of this, and in flagrant contravention of scientific method, Jost's results were accepted and incorporated into subsequent research designs, because of cultural notions of male and female within western capitalist society.

With the historical perspective in mind, the current level of scientific understanding of embryonic development and hormonal influences will be discussed. As will be demonstrated, there is a serious discrepancy in the level of understanding of female and male development.

EMBRYONIC DEVELOPMENT OF THE UNDIFFERENTIATED GONADS

At an early stage in the developing embryo, the germ cells originating in the yolk sac migrate to the genital ridges (Wilson, George and Griffin 1981:1278). The undifferentiated gonad in the male and female at this time are identical, consisting of the primordial germ cells, the interstitial cells, and a surrounding layer of epithelium (Wilson, George and Griffin 1981:1278). These three cell types are destined to serve the same sex-specific functions in the female and male gonad respectively. The primordial germ cells in the testes become sperm and the germ cells of the ovaries become ova (Jost and Magre 1988:56). The interstitial cells form the follicle envelopes in the female and Sertoli cells in the male, as well as the hormone secreting cells (Burgoyne 1988:64).

Germ cells require an appropriate gonadal milieu to elicit the formation of functional gametes (Haseltine and Ohno 1981:1273). The subsequent genital development of females and males follow separate routes.

The male begins to emerge early in the zygote development, as evidenced by the presence of rudimentary seminiferous tubules and subsequent somatic cell differentiation (Burgoyne 1988:63, 64). In testicular development, the Sertoli cells aggregate around the germ cells at an early time, and are believed to cause the differentiation of the Leydig cells (Jost and Magre 1988:56). The Leydig cells secrete testosterone, resulting in the continued development of the rudimentary testes along the testicular developmental pathway (Burgoyne 1988:64).

The primordial ovaries, on the other hand, exhibit very little differentiation until late in development, when the follicles begin to

emerge prenatally (Wilson, George and Griffin 1981:1279) and have completely differentiated postnatally (Jost and Magre 1988:56). It is interesting to note, however, that the ovary begins to secrete estrogens at the same time the testes begin to secretion testosterone (i.e., beginning at about 30 days of gestation for humans) (Wilson, George and Griffin 1981:1279). Thus, biochemical differentiation of the testes and ovaries is contemporaneous.

The two networks of female and male specific ducts develop concurrent with the development of the gonads; in fact, both develop together, initially, and eventually one or the other remains (for clarity, refer to Figure 1). In the female, the Mullerian ducts remain, and the Wolffian ducts regress (Wilson, George and Griffin 1981:1280). The Mullerian ducts mature to form the fallopian tubes, the uterus and the upper vagina (Eicher and Washburn 1986:329). In the male, the Wolffian ducts mature to form the epididymis, the vas deferens and the seminal vesicles (Eicher and Washburn 1986:329) and the Mullerian ducts regress in response to Mullerian Inhibition Factor, which is released by the testes (Wilson, George and Griffin 1981:1280). There is evidence that the Wolffian ducts are further developed by the action of testosterone (ibid). Interestingly, and in contrast to this, no studies have been carried out to determine whether the ovaries are the primary inducers of the Mullerian duct network in the female embryo (Eicher and Washburn 1986:329).

In fact, tracing the morphological events currently believed to lead to and include gonadal differentiation, the notion of female passivity is implicit. Accordingly, differentiation is defined as having started only when the testes begin to form the rudimentary spermatogenic cords (Jost and Magre 1988:57). Indeed, during fetal development, ovaries are characterized as gonads lacking in testicular structures (Burgoyne 1988:63), even though hormone-secreting cells in females and males only begin to function after differentiation. Rather than assessing biochemical differentiation, which occurs simultaneously in developing female and male embryos (Wilson, George and Griffin 1981:1279), researchers have chosen to use gross morphology and histology to indicate gonadal differentiation. While this is undoubtedly a consequence of the assumption of 'female passivity', it is also a reflection of the current state of knowledge concerning the ovary. Very little research has been done on ovarian development (Eicher and Washburn 1986:329), a telling example of androcentric bias in sex determination work.

HORMONAL INFLUENCES ON SEX DIFFERENTIATION

The lack of research on females does not stop with ovarian origins, but continues in the area of hormonal influences on the developing female embryo. Almost all sexual dimorphism is explained as a result of the action of testosterone, or the lack of it (cf Bardin and Catterall 1981). For example, one review concerning hormonal influences states the following:

> The burden of sex differentiation falls on the testes. The testes must be formed early and masculinizing hormones must be produced very early in development (Haseltine and Ohno 1981:1274)

Consistent with this view, testosterone secretion by the Leydig cells is said to promote maturation of the spermatogenic tubules and to contribute to the formation of the male genital tract; a derivative of testosterone, 5-dihydrotestosterone, is responsible for the formation of the external genitalia (Wilson, George and Griffin 1981:1280). However, estrogens produced by the ovary are thought to play no role in the differentiation of the developing embryo (Haseltine and Ohno 1981:1274), despite the fact there is a vacuum of knowledge concerning the hormonal influences governing the developing ovaries (Eicher and Washburn 1986:329; Wilson, George and Griffin 1981:1274).

Once again, the paradigm of passive female development emerges, in contrast to male development where a battle is waged to diverge from the female path.

The androcentric bias and idea of female passivity is overtly rationalized as follows:

> Investigators have mainly studied the existence of masculinizing substances since testicular differentiation appears to be under a more direct embryonic control than ovarian development (Haseltine and Ohno 1981:1274).

The assumption that testicular differentiation is under "more embryonic control" than ovarian differentiation is clearly a consequence of cultural notions of female passivity; hence, femaleness is the natural or "default" route. That is to say, there is an insistence on the part of researchers to find a gene that acts to induce testicular development. This gene, labelled testes determining factor (TDF) represents the molecule of determination, the molecule of action, the molecule of masculinity.

The pursuit of maleness, embodied in the search for testicular determination, has also been pursued at the chromosomal level in parallel with the investigations of the physiological route.

THE Y CHROMOSOME

In 1959, Jacobs and Strong reported the first ever human chromosomal abnormality (Jacobs and Strong 1959:302). The individual was a phenotypic male with a sex chromosome karyotype of XXY, rather than the normal XY karyotype. Immediately thereafter, several researchers reported other human sex chromosomal abnormalities (cf McLaren, 1988 for a review). The early research noted that the presence of a Y chromosome was consistently associated with a male phenotype, prompting Welshons and Russell (1959:565) to hypothesise the male-determining properties of the Y chromosome. Indeed, to date, there appears to be no direct evidence to contradict this hypothesis (Eicher and Washburn 1986:331).

It is generally thought that one or more genes on the Y chromosome pre-empt ovarian development (Burgoyne 1988:63) by initiating the earlier development of the testes. One gene product thought to be transcribed from the Y chromosome was H-Y antigen.

H-Y ANTIGEN

In 1955, it was found that inbred female mice reject male skin grafts of their litter mates (Eichwald and Silmser 1955:149). It was only much later that this was explained in terms of a male-specific histocompatibility antigen designated H-Y (see Goldberg, 1988 for a review). The result of the discovery of a serum detected male antigen (designated SDMA) (Goldberg, Boyse, Bennett et al. 1971:479), as opposed to transplantation detected male antigen (H-Y) (Eichwald and Silmser 1955:149), opened the gates to many interesting experiments, since they could now be conducted *in vitro* under controlled conditions.

It was soon found that SDMA was phylogenetically conserved in XY males of the rabbit, rat, guinea pig as well as humans (Goldberg 1988:74). As this evolutionary conservation implied an important function, it was suggested that H-Y antigen represented the much-sought testes determining factor (Wachtel, Ohno, Koo et al. 1975:236). Following the evidence that desegregated rodent testicular cells coalesced into structures superficially resembling seminiferous tubules (Goldberg 1988:76), it was

found that this reassociation was blocked by the addition of H-Y specific antibody (Goldberg 1988:76). Moreover, SDMA was detected as early as the eight cell stage of male embryonic development, further implicating it in its putative role as TDF (Haseltine and Ohno 1981:1275). H-Y antigen antiserum was also found to kill 50% of developing mouse embryos, postulated to be males (although not tested) (Haseltine and Ohno 1981:1275). That is, the block of H-Y antigen was thought not to allow normal male development to continue.

It has been clearly demonstrated that H-Y antigen is not required for testicular differentiation in mice (McLaren, Simpson, Tomonari et al. 1984:554). Evidence discounting the possible role of H-Y antigen as TDF emerged from the separation of the chromosomal location of the mythical TDF from H-Y antigen (Simpson, Chandler, Goulmy et al. 1987:877). Specifically, the two genes reside on different loci on the Y chromosome.

Indeed, the finding of XX individuals, phenotypic males without a Y chromosome, has led to the search for relevant genes on the Y chromosome required for male development. It has not led to abandonment of the molecular pursuit of masculinity.

THE MAMMALIAN Y CHROMOSOME: MOLECULAR STUDIES

It is postulated that during meiosis there is an obligatory pairing of homologous chromosomes to allow for proper segregation of the correct complement of genetic information to the respective division products (Earnshaw, Halligan, Cooke, et al. 1985:1713). Even though the Y chromosome was thought to have no equivalent homologue as does the X chromosome in females, as early as 1934 it was known that the X and Y chromosomes pair during meiosis (Goodfellow, Darling, Thomas et al. 1986:740). Moreover, we now know that there is an obligatory crossing-over event between the short arm of the X and Y chromosomes during male meiosis (Weissenbach, Levilliers, Petit et al. 1987:68). This region of crossing-over is referred to as the pseudo-autosomal region (Goodfellow, Darling, Thomas et al. 1986:740). As this crossing-over event may not always proceed properly, many XX males have been found to harbour Y specific sequences, as well as XY females missing Y chromosomal material who, nevertheless, maintain a normal female phenotype (Ferguson-Smith and Affara 1988:134,135). Clearly, human X and Y chromosomes are homologous (Page, Harper, Love et al. 1984:122).

The realization that some XX males harbour Y chromosomal specific DNA and that XY females are missing some of the Y chromosome has allowed the construction of a deletion map of the Y chromosome (Ferguson-Smith, Affara and Magenis 1987:46). This was executed in

order to attempt to localize the gene for TDF more accurately on the Y chromosome.

THE MAPPING OF TDF ON THE Y CHROMOSOME

In light of the homology of the human X and Y chromosome (Page, Harper, Love et al. 1984:122), abnormal chromosomal crossing over seems to occur at a high frequency of about 1 in 20000 meiosis (Andersson, Page and De La Chapelle 1986:787). In other words, abnormal crossing-over events may exceed the pseudoautosomal region and include sections of the Y chromosome not included in this region. By identifying individuals with this genetic make-up, it was found that TDF maps to about 2% to 3% map units of the pseudo-autosomal region (Goodfellow, Darling, Thomas et al. 1986:740). These data were further substantiated by the serendipitous finding of a strain of mice that exhibit a male phenotype, despite the presence of a sex karyotype of XX (cf Eicher and Washburn, 1986 for a review). This condition is called sex reversal.

The sex reversed condition in mice (designated Sxr) is a consequence of a rearranged Y chromosome with a translocation of the region bearing the testes determining function onto the tip of the long arm of the Y chromosome (McLaren 1988:4). (Refer to figure 2 for clarification.) Because the new location of this duplication is beyond the pseudoautosomal region of the Y chromosome, it is able to transfer onto the X chromosome during male meiosis (McLaren 1988:4). This can give rise to X chromosome bearing sperm with this anomaly. Fertilization of an ovum by this variety of sperm would result in an XX male mouse.

In recent years, several technological advances have permitted molecular geneticists to isolate any gene in the genome without any prior knowledge of its structure. This technique hinges on the ability to map, precisely, the location of the gene. This is exactly what was done with TDF, taking the search for maleness to an increasingly finer level of analysis.

ZFY AND ZFX

Using deletion studies of sex-reversed individuals, a small (230 kilobase pairs) portion of the Y chromosome has been identified (called "interval 1") as necessary and sufficient to induce testicular determination of the indifferent, bipotential gonad (Page, Mosher, Simpson et al. 1987:1092). By sequencing the area, it was found to contain a DNA binding protein (designated ZFY). ZFY also exists in all mammals tested.

Interestingly, a homologue for ZFY was found on the X chromosome of all mammals tested, called ZFX (Page, Mosher, Simpson et al. 1987:1095). It was thought that ZFX is inactivated and thus sexual determination is reduced to the dosage effect of this one gene (Page, Mosher, Simpson et al. 1987:1101). Although this has subsequently proven to be incorrect, once again, "females are females through a certain incapacity" (Agonito 1977:46). That is, the incapacity of ZFX to produce the ZFX gene product was suggested before any experiments had been executed.

Further characterization of ZFY and ZFX has revealed that neither is inactivated and that they have only ten amino acid differences (Schneider-Gadicke, Beer-Romero, Brown et al. 1989:1251). Thus, it is very likely that they are both able to bind to the same DNA sequences (Schneider-Gadicke, Beer-Romero, Brown et al. 1989:1254). No substantial explanation has been put forward to account for the similarities between ZFX and ZFY, (Schneider-Gadicke, Beer-Romero, Brown et al. 1989:1255, 1256).

Despite the initial acceptance of ZFY as the testes determinant, several researchers have found evidence to discount it as the primary trigger of sexual determination. Specifically, four human XX males lacking a copy of ZFY were found to harbour Y specific DNA next to the pseudoautosomal region (Palmer, Sinclair, Berta et al. 1989:938). These results suggest that the location of TDF must be redefined.

The evidence that ZFY is not in all males, as well as the fact that ZFY has a homologue on the X chromosome (ZFX), has not resulted in a new outlook. Rather, the researchers who found this anomaly merely maintain that the mythical TDF gene is somewhere else on the Y chromosome (Palmer, Sinclair, Berta et al. 1989:939). Fortunately, some researchers are losing their myopia and have begun to look to other aspects of the genome in the pursuit of sex determination. Recent work has explored the role of autosomal genes in sex determination.

AUTOSOMAL GENES INVOLVED IN SEX DETERMINATION

Despite the insistence on the Y chromosome as the determinant of the male phenotype, there is a growing body of evidence that implies that autosomal genes are recruited, not only in testicular and ovarian differentiation, but also in their determination. It was found that when the Y chromosome of *Mus domesticus* was transferred onto another mouse strain (called C57BL/6J), XY mice developed as females with ovaries (Eicher, Washburn, Whitney et al. 1982:536). In an attempt to explain this

bizarre finding, it was suggested that the C57BL/6J genetic background contains an autosomal allele (designated testes determining autosomal-1, Tda-1) that causes ovarian tissue to develop in XY mice (Eicher, Washburn, Whitney et al. 1982:537).

Further evidence for the involvement of autosomal genes in sexual determination come from studies of human XX males as well as human XX true hermaphrodites. It appears that 31% of XX males studied have no detectable Y chromosome specific DNA (De La Chapelle 1987:34). Several instances are documented in which XX males had no detectable Y DNA but were subsequently found to contain Y specific sequences (De La Chapelle 1987:34). To date there is no evidence of Y sequences in XX true hermaphrodites (De La Chapelle 1987:34).

This has prompted one researcher to suggest that testicular determination may occur through a mechanism not requiring TDF (De La Chapelle 1987:36). While he concedes that these individuals may have only a small fraction of the cells having Y chromosomal DNA, another possibility that is recognized has more far reaching implications. This second suggestion calls for an autosomal or X linked gene that mutates into one that controls testicular determination (De La Chapelle 1987:36). The available evidence suggests that this putative gene acts as an autosomal dominant mutation referred to as TDFA (i.e., TDF-Autosomal) (De La Chapelle 1987:36). TDFA is now believed to act in the same manner as the fabled TDF.

DISCUSSION

The establishment of a paradigm is typically precipitated by a sufficiently unprecedented discovery that attracts followers and is open ended enough to allow further research (Kuhn 1970:10). The original experiments of Alfred Jost and the interpretation of his findings have been instrumental in the androcentrism surrounding the study of sex determination. Just as Aristotle suggested that females lack the capacity to concoct sperm (Agonito 1977:46), the single-factor hypothesis paradigm of sex determination developed by Jost stimulated subsequent research on single factors. Furthermore, each single factor postulated has been a male specific phenomenon, something that females are missing. As scientific technology improved, it merely served to lend increasing specificity to the etiology of maleness. Thus, maleness was first thought to be a consequence of testicles (McLaren 1988:4); subsequently, male development and determination was thought to be caused by testosterone (Wilson, George and Griffin 1981:1280), the Y chromosome (Eicher and

Washburn 1986:330, 331), H-Y antigen (Wachtel, Ohno, Koo et al. 1975:236) and, finally, ZFY (Page, Mosher, Simpson et al. 1987:1100).

The insistence on a single factor explanation for this complex phenomenon has resulted in several anomalies. First, there is no good explanation for XX hermaphrodites. Second, as all the single factors explored to date are male-specific, there is virtually no knowledge of the factors affecting female determination. Indeed, if "the term determination is used in embryogenesis to indicate irreversible commitment to a particular developmental pathway" (Erickson and Durbin 1987:25), then *ipso facto* female determination cannot be studied if "irreversible commitment" is already present. A similar tautology is established by defining the primary sexual characteristics as the presence of gonads and the secondary sexual characteristics as consequences of the primary. The result is that the female is completely ignored and female hormones are not studied at the developmental level (Eicher and Washburn 1986:329).

It is evident that some researchers tend to ignore anomalies in an effort to ensure that their observations reflect and confirm the prevailing constructed view of reality. The inability of the current androcentric paradigm is only one such example. Kuhn (1970) explains this phenomenon as follows:

> No part of the aim of normal science is to call forth new sorts of phenomena; indeed those that will not fit the [paradigm] are often not seen at all ... Instead, normal-scientific research is directed to the articulation of those phenomena and theories that the paradigm already supplies (Kuhn 1970:24).

More recently, Hubbard (1990:209) has suggested that biology is profoundly political. Indeed, "to be believed, scientific facts must fit the world view of the times" (Hubbard 1990:25).

It is clear that the interactions involved in sex determination are of a nature more subtle than presently realized. Thus, single factorial explanations concentrating on male-specific elements are inadequate. A multifactorial approach involving autosomal gene interactions and female developmental factors must be undertaken. This can only be achieved by placing a greater emphasis on the study of female embryonic development. In doing so, light will be shed on the manner in which female development is controlled. Only then will an egalitarian science of developmental biology result.

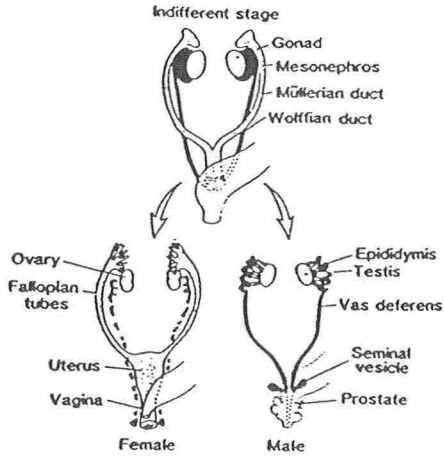

Figure 1. The structure of the female and male internal ducts. Taken from Wilson, George and Griffin 1981:1280.

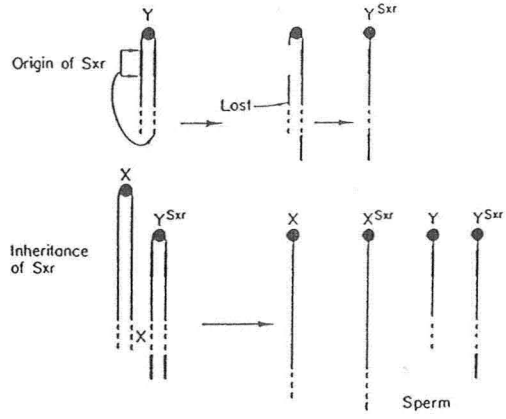

Figure 2. The structure of the Sxr Y chromosome and the four chromosomal products resulting from crossing-over. Taken from Eicher and Washburn 1986:349.