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# Science Watchers

# Mammalian Sex Determination

**Mark S. Palmer**

TWO RECENT reports in *Nature* [1, 2] have suggested that the elusive sex-determining gene (or more properly that for the testis determining factor [*TDF*]) may finally have been identified, with the description of a new gene, *SRY*, from the smallest region of the human Y chromosome known to be sex-determining (hence sex-determining region on the y chromosome). The history of the quest for *TDF* has been somewhat chequered, and *SRY* is the fourth candidate sequence to be described. It is therefore understandable that the two groups of investigators have stressed that while *SRY* is now the best candidate sequence, the proof must await functional studies of sex-reversed transgenic mice or mutational analysis of XY females. Indeed the possibility of other coding sequences near to *SRY* has not been ruled out.

The Y chromosome has been known to be sex-determining in man since 1959 following observations on the karyotype of a male with Klinefelter syndrome [3] and a female with Turner syndrome [4]. The Y chromosome has since been subjected to extensive genetic and molecular analysis in an attempt to pinpoint the genes involved. An early sequence thought to play a role in sex-determination was Bkm (banded krait mini satellite) DNA, a satellite DNA containing mostly simple GATA–GACA tandem repeats. Bkm sequences, as well as showing sex-specific localisation in the snake, were concentrated on the mouse Y chromosome, particularly on the sex-determining region (*SXR*); however, they were poorly represented on the human Y chromosome [5]. This left the stage open for the most enduring candidate, the male-specific minor histocompatability antigen, H-Y, originally described as a male-specific transplantation antigen on male skin grafts in certain strains of inbred mice. The most fundamental requirement of a sex-determining gene on the Y chromosome is that its presence should be necessary for the development of the indifferent fetal gonad into a testis. (After testicular development has been initiated further sexual development is under hormonal control.) The finding of male mice and men with normal testicular development but lacking H-Y antigen meant that this hypothesis also had to be abandoned [6, 7].

The next sequence to be considered, *ZFY*, was identified by a molecular analysis of that part of the Y chromosome inherited by XX males. Ordinarily during male meiosis the X and Y chromosome pair and undergo an obligatory recombination

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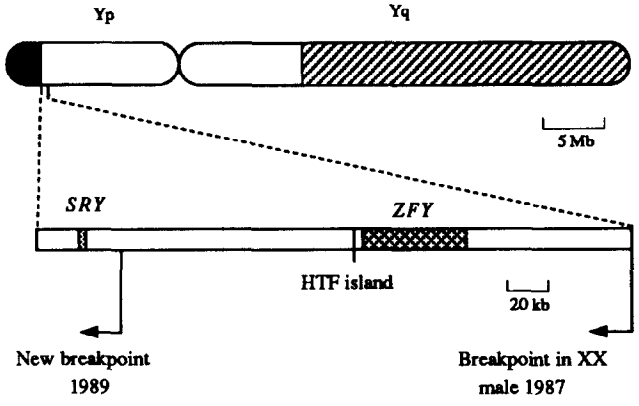


**Fig. 1. Y chromosome expanded to show the positions of *ZFY* and *SRY* within 280 kb. Shading on long arm (Yq) is heterochromatic DNA. 2.5 Mb psuedoautosomal region is shown in black on tip of short arm (Yp).**

event within the region that they both share on the tip of their short arms. The recombination is restricted to this pseudoautosomal region to maintain the chromosomal basis of sex determination. However, occasionally the recombination event occurs outside the pseudoautosomal region and Y-specific sequences are transferred to the X chromosome. If this X chromosome is inherited then the XX individual develops as a male, with testes, due to the Y-specific sequences containing the *TDF* gene. The breakpoints for recombination in XX males are clustered at a number of sites along the Y chromosome. In 1987 an XX male was described with the smallest segment of the Y then known, just 280 kb of DNA proximal to the pseudoautosomal boundary (Fig. 1) [8]. Of this, 140 kb overlapped with a deletion in a sex-reversed XY female who had a Y;22 translocation. Within the 140 kb overlap a new exon, encoding a domain of 13 zinc-finger motifs, was found and called *ZFY* for zinc finger on the Y chromosome. *ZFY* was certainly a strong candidate for *TDF*. It was positioned at the first *Hpa* II tiny fragment (HTF) island proximal to the pseudoautosomal boundary, at a site previously predicted to encode *TDF*, and its deletion in the X t(Y;22) female would explain sex reversal in this individual. The characteristics of the encoded protein, potentially a DNA-binding protein with a transcriptional activation domain [9], were also strong indicators that *ZFY* might play a role in gene regulation, a function consistent with the expected action of *TDF*. Less consistent with *ZFY* being *TDF* was the presence of a homologous gene on the X chromosome, *ZFX*, and the absence of *ZFY* from the sex chromosomes of marsupials [10], in which the Y chromosome is also sex-determining.

In December 1989, four XX sex-reversed individuals were described who lacked *ZFY* [11]. These individuals (three XX males and one true hermaphrodite) possessed an even smaller fragment of Y-specific material, now determined to be just 35 kb. The absence of *ZFY* in these males excluded *ZFY* as a candidate for *TDF*, and predicted that a conserved gene sequence would be contained within the small fragment. It is within this region that *SRY* has been identified by the exhaustive mapping of cloned sequences against panels of male and female DNA [1]. The gene, about 1 kb in size, contains within its conceptual open reading frame an 80 aminoacid motif with homology to a motif found in high mobility group proteins (the HMG-box), a human RNA Pol I transcription factor, and also to the Mc protein of the yeast *Schizosaccharomyces pombe*, known to be involved in yeast mating type and meiosis. The *SRY* motif, possibly a conserved type of DNA-binding domain, has been found on the Y chromosome of several eutherian mammals, and the Y chromosome sequence has been determined in rabbits and mice. The mouse *SRY* gene also maps to the smallest sex-determining region known (*SXR'*) and was deleted in an XY female mouse [2]. This deletion is believed to be small, and if it does not extend greatly beyond the sequence of *SRY* will be strong evidence for the equivalence of *SRY* and *TDF*. Interestingly, the translocated Y chromosome of the X t(Y;22) woman who had been used to locate *ZFY* has an additional deletion of 50 kb across the region containing *SRY* [12].

Perhaps of more general interest is the finding that the HMG-box motif of *SRY* cross-reacts with several independent mouse cDNAs, but only in libraries prepared from early embryos [2]. While this sequence clearly defines a new family of related genes, it would be exciting if their expression is restricted to the early stages of development, especially if the family does have a regulatory function in gene expression. Not only can the unravelling of the genetic pathway of sex determination in mammals now commence, providing that *SRY* maintains its promise of being equivalent to *TDF*, but a whole new window on the genetic control of developmental processes may also have been opened.


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