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Is *ZFY* the sex-determining gene on the human Y chromosome?

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The sex-determining region of the human Y chromosome contains a gene, *ZFY*, that encodes a zinc-finger protein. *ZFY* may prove to be the testis-determining factor. There is a closely related gene, *ZFX*, on the human X chromosome. In most species of placental mammals, we detect two *ZFY*-related loci: one on the Y chromosome and one on the X chromosome. However, there are four *ZFY*-homologous loci in mouse: *Zfy-1* and *Zfy-2* map to the sex-determining region of the mouse Y chromosome, *Zfx* is on the mouse X chromosome, and a fourth locus is autosomal.

Studies of humans and mice with abnormal sex-chromosome constitutions have revealed the critical sex-determining role of the Y chromosome (reviewed by McLaren, this symposium). Regardless of the number of X chromosomes, human or mouse embryos with a Y chromosome (XY or XXY) develop as males, with testes, whereas embryos with no Y chromosome (X0 or XX) develop as females, with ovaries. These findings imply the existence on the Y chromosome of one or more genes whose products determine, directly or indirectly, the fate of all sexually dimorphic characters.

To facilitate discussion of these inferred but uncharacterized sex-determining gene or genes on the Y chromosome, they have been given names. Thus in complete ignorance of the biochemical nature, mode of action or even the number of gene products, one can refer abstractly to the *TDF* (testis-determining factor; McKusick (1975)) gene(s) on the human Y chromosome or to *Tdy* (Y-linked testis determinant; Eicher *et al.* (1982)), the murine counterpart.

My co-workers and I set out to identify the human *TDF* gene(s) by an approach that does not presuppose the nature of the gene product(s). We thought it would be possible to clone the gene by determining its precise location on the Y chromosome (Page 1986). A deletion map of the human Y chromosome can be constructed by DNA hybridization analysis of naturally occurring, structurally abnormal Y chromosomes (Vergnaud *et al.* 1986), and *TDF* can be positioned on such a map. By genetic deletion analysis of 'sex-reversed' individuals (e.g. 'XX males' and 'XY females'), we established that the fate of the bipotential gonad hinges upon the presence or absence of a very small portion of the short arm of the Y chromosome (Page *et al.* 1987). Indeed, testicular differentiation occurred in an XX male who carries roughly 300 kilobase pairs (intervals 1A1 and 1A2), or 0.5%, of the Y chromosome (figure 1). Conversely, female differentiation occurred in an individual who apparently possesses all but 160 kilobase pairs (intervals 1A2 and 1B) of the Y chromosome. Deletion analysis of these and other individuals suggests the following two conclusions: first, interval 1A (the sum of 1A1 and 1A2) is sufficient to induce testicular differentiation of the bipotential gonad; second, interval 1A2 contains an essential portion of that testis-determining function.

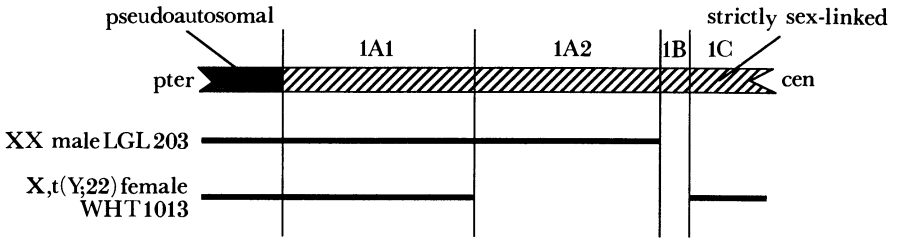

FIGURE 1. The sex-determining region of the human Y chromosome (adapted from Page *et al.* (1987)). The distal short arm of the Y chromosome is represented schematically, oriented with respect to the short-arm telomere (pter) and centromere (cen). The pseudoautosomal region frequently undergoes recombination with the X chromosome during meiosis. Intervals 1A1, 1A2, 1B and 1C show strictly sex-linked (not pseudoautosomal) inheritance, and they are defined by deletion analysis. Black bars depict the portions of the Y chromosome present in XX male LGL203 and X, t(Y; 22) female WHT1013. The *TDF* gene must be found in its entirety in intervals 1A1 and 1A2. Interval 1A2, which is present in the XX male and absent in the X, t(Y; 22) female, must contain an essential portion of *TDF*.

What gene or genes are actually found in interval 1A2, which measures 140 kilobase pairs, or about 0.2 % of the human Y chromosome? We discovered that interval 1A2 carries a gene that, based on analysis of its nucleotide sequence, appears to encode a protein with at least 13 'zinc-finger' domains (Page *et al.* 1987). The presence of zinc-finger domains, as first described in frog transcription factor IIIA (reviewed by Klug & Rhodes (1987)), suggests that the putative protein binds to DNA or RNA in a sequence-specific manner. The protein may regulate transcription.

The location of this gene in interval 1A2 – and the existence of homologous sequences in the *Sxr* region of the mouse Y chromosome, as described below – suggests that this zinc-finger protein is sex determining. However, in the absence of more direct evidence of sex-determining function (e.g. sex reversal of transgenic XX mice, or the finding of a mutation within the gene in an XY female), it is premature to refer to the gene as *TDF*. Until its biological function is determined, I shall refer to the human gene simply as *ZFY* (Y-linked zinc-finger protein).

Surprisingly, there appears to exist on the short arm of the human X chromosome a gene whose structure and DNA sequence are quite similar to those of *ZFY* (Page *et al.* (1987) and unpublished results). Until the biological function of this gene is established, I shall refer to it as *ZFX* (X-linked homologue of *ZFY*). Although it is quite likely that *ZFY* and *ZFX* are true homologues that evolved from a single, common ancestral gene, it is unlikely that either is a pseudogene, for both show a striking degree of evolutionary conservation among placental mammals (Page *et al.* 1987). If *ZFY* is the Y-linked sex-determining factor then we must consider models of sex determination that accommodate the existence of a related gene on the X chromosome (see Page *et al.* (1987) for a discussion of four such models).

It should be noted that interval 1A2 of the Y chromosome is absent in some human XX males and XX hermaphrodites, and it is at least grossly intact in many XY females (Page *et al.* 1987). Sex reversal in some such cases may be due to mutations in autosomal or X-linked genes whose products function together with or downstream of *TDF* in the sex determination pathway.

In most species of placental mammals that we have examined, we have detected two loci homologous to human *ZFY*: one on the Y chromosome and one on the X chromosome. However, mice appear to have four loci homologous to human *ZFY* (Page *et al.* (1987) and unpublished results). One of these homologues is on the mouse X chromosome, and I shall refer to it as *Zfx* (X-linked homologue of human *ZFY*). The mouse Y chromosome carries two

distinct loci homologous to human *ZFY*. I shall refer to these mouse loci as *Zfy-1* and *Zfy-2* (Y-linked homologues of human *ZFY*). The fourth mouse locus is autosomal.

Adding to the evidence that *ZFY* functions in human sex determination is the finding (Page *et al.* 1987) that both *Zfy-1* and *Zfy-2* are present in XX *Sxr* male mice, which carry only a small, sex-determining portion of the mouse Y chromosome (Singh & Jones 1982; Evans *et al.* 1982). Interestingly, *Zfy-1* is present but *Zfy-2* is absent (G. Mardon, unpublished results) in XX *Sxr′* male mice (McLaren *et al.* 1984), who carry an even smaller but none the less sex-determining portion of the mouse Y. *Zfy-1* and *Zfy-2* evidently are not both necessary for testis determination.

Detailed examination of the human *ZFY* and *ZFX* genes, the mouse homologues and the encoded proteins is clearly warranted. None the less, interval 1A1 and the remainder of interval 1A2 of the human Y chromosome merit further scrutiny. The possibility that this sex-determining region contains one or more genes in addition to *ZFY* cannot yet be excluded.

References

Eicher, E. M., Washburn, L. L., Whitney, J. B. III & Morrow, K. E. 1982 *Mus poschiavinus* Y chromosome in the C57BL/6J murine genome causes sex reversal. *Science, Wash.* **217**, 535–537.

Evans, E. P., Burtenshaw, M. D. & Cattanach, B. M. 1982 Meiotic crossing-over between the X and Y chromosomes of male mice carrying the sex-reversing (Sxr) factor. *Nature, Lond.* **300**, 443–445.

Klug, A. & Rhodes, D. 1987 "Zinc fingers": a novel protein motif for nucleic acid recognition. *Trends biochem. Sci.* **12**, 464–469.

McKusick, V. A. 1975 *Mendelian inheritance in man*, 4th edn. Baltimore: Johns Hopkins University.

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

Page, D. C. 1986 Sex reversal: deletion mapping the male-determining function of the human Y chromosome. In *Molecular biology of Homo sapiens* (*Cold Spring Harb. Symp. quant. Biol.* **51**), pp. 229–235.

Page, D. C., Mosher, R., Simpson, E. M., Fisher, E. M. C., Mardon, G., Pollack, J., McGillivray, B., de la Chapelle, A. & Brown, L. G. 1987 The sex-determining region of the human Y chromosome encodes a finger protein. *Cell* **51**, 1091–1104.

Singh, L. & Jones, K. W. 1982 Sex reversal in the mouse (*Mus musculus*) is caused by a recurrent nonreciprocal crossover involving the X and an aberrant Y chromosome. *Cell* **28**, 205–216.

Vergnaud, G., Page, D. C., Simmler, M.-C., 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.