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Mapping of testis-determining locus on Yp by the molecular genetic analysis of XX males and XY females

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Summary

Sex reversal in males with female karyotypes is likely to be caused by the presence of cytogenetically undetectable Y-chromosomal DNA sequences that include the testis-determining gene(s). Studying a total of sixteen 46,XX males and one 47,XXX male, we detected Y-chromosomal DNA in 13 of the XX males (i.e. 80 %) and in the 47,XXX male. The amount of Y-chromosomal DNA present in the patients varied between individuals. This allowed the construction of a molecular map of the Y-chromosome short arm. The putative testis-determining locus was assigned to the more distal portion of Yp, yet proximal to the pseudoautosomal region. Mapping of the testis-determining locus was complemented by molecular findings in 46,XY females. These individuals may carry microdeletions of the portion of Yp that appears to be required for normal male gonadogenesis. The deletions detected in 46,XY females always included those Y-chromosomal DNA sequences that were found in most 46,XX males. Furthermore, the same DNA sequences were missing in a female with a 46,X,dic(Y) karyotype. The observations suggest that some of our DNA probes hybridize with Y-chromosomal DNA sequences within a few million base pairs of the testis locus. Chromosome walking and pulsed-field gel electrophoresis investigations have been initiated in order to isolate those Y-chromosomal DNA sequences that are required for normal testicular development.

Key words: Y-chromosomal DNA probes, testis differentiation, sex reversal, XX male, XY female.

Introduction

The close correlation between the presence of a Y chromosome and a male phenotype suggests that there are gene(s) located on the Y that determine male sex differentiation in mammals. Such gene(s) are likely to be involved in early events of testicular development and may trigger the differentiation of the male gonad from an anlage that is morphologically indistinguishable in the prospective male and female. Given that Sertoli cells are the first testicular cell type to be distinguished morphologically (Jost, Magre & Agelopoulou, 1981), Y-chromosomal sex-determining gene(s) may be involved in the differentiation of these cells. As yet, however, nothing is known about the nature of these gene(s). The following article delineates the possible location of testis-determining gene(s) on the human Y chromosome and describes approaches towards their eventual isolation.

Isolation of DNA sequences from the euchromatic portion of the Y

Cytogenetic findings have shown that the human Y chromosome consists of an euchromatic and of a heterochromatic portion (Caspersson, Lomakka & Zech, 1971; Schempp & Müller, 1982). The former comprises the entire short (Yp) and the proximal long arm (Yq); the latter the centromeric region and the distal long arm. The occurrence in normal fertile males of Y chromosomes lacking the heterochromatic portion of Yq (Verma, Dosik, Scharf & Lubs, 1978) suggests that this portion does not contain genes required for male development and function. Such genes appear to be confined to the euchromatic portion of the Y (Bühler, 1980). This portion may comprise 30 % to 40 % of the Y-chromosomal DNA content (Cooke, Fantes & Green, 1983). Assuming that the entire Y contains 60×10^6 base pairs (bp),

functional genes are confined to $18–24\times10^6$ bp of Y-chromosomal DNA.

A first step towards the eventual detection of Y-chromosomal testis-determining gene(s) is the isolation of DNA sequences from the Y euchromatic portion. Therefore, we have constructed a recombinant DNA library from flow-sorted human Y chromosomes (Müller, Lalande, Disteche & Latt, 1986*b*). The DNA extracted from 5×10^6 Y chromosomes was cleaved with restriction enzyme *Hin*dIII and cloned into the *Hin*dIII sites of the lambda phage vector Charon 21A. This vector has a maximum acceptable insert size of 9 kbp. Since the heterochromatic portion of distal Yq consists of highly repeated satellite DNA virtually lacking *Hin*dIII sites (Cooke, Scmidtke & Gosden, 1982), most of this DNA was not clonable. As a result, the library was enriched for DNA from the Y euchromatic portion. In fact, of approximately 20 Y-chromosomal DNA sequences analysed, none was derived from distal Yq (unpublished findings).

The probes isolated from the library could be assigned to one of five groups according to their hybridization pattern. Most of them either hybridized with a Y-specific restriction fragment in addition to repeated non-Y chromosomal sequences (group IV of Müller *et al.* 1986*b*) or detected repeated sequences that are present to a greater extent in DNA from Y-containing cell lines than in DNA from cells lacking a Y (group V). These probes were not used in further studies since they yield complex hybridization patterns and are not useful either for chromosome walking or for analysis using the recently described pulsed-field electrophoresis techniques (Schwartz & Cantor, 1984; Carle, Frank & Olson, 1986). Probes assigned to groups I–III which hybridize with a Y-specific single-copy DNA sequence, with Y-specific repeated sequences, or with a single-copy Y-chromosomal DNA sequence in addition to a single-copy X or autosomal DNA sequence (Müller *et al.* 1986*b*) are more suitable for further molecular genetic studies. Some of these probes are listed in Table 1.

Y-chromosomal DNA sequences in XX males and XY females

Several mutations that interfere with normal gonadogenesis are useful for studying the testis-determining locus in the euchromatic portion of the Y chromosome. These mutations include the XX male and the XY female (Swyer's) syndrome in man. The former describes normal but infertile males with a 46,XX (de la Chapelle, 1981) or, very rarely, a 47,XXX (Bigozzi *et al.* 1980) karyotype. The latter syndrome is characterized by a lack of testis differentiation in patients with a 46,XY karyotype and a resulting female phenotype (Swyer, 1955; Simpson, Blagowidow & Martin, 1981).

XX males

Cytogenetic findings suggest that certain XX males may carry Y-chromosomal material on the tip of the short arm of one of their X chromosomes (Madan & Walker, 1974; Wachtel *et al.* 1976; Magenis *et al.* 1982). This was interpreted according to the hypothesis of Ferguson-Smith (1966) as evidence of unequal X–Y interchange during paternal meiosis which resulted in the insertion of Y-chromosomal DNA (including testis-determining gene(s)) in a 46,XX zygote.

Molecular studies using Y-specific DNA probes have now shown Y-chromosomal DNA sequences

Table 1. *Y chromosomal DNA probes referred to in this article*

Characteristics	Probe description	Probe size (kb)	Localization on Y
Y-specific single copy	Y-202	1·5	Yq11 21→Yq11.23
	Y-214	2·3	Yq11.21→Yq11.23
	Y-253	3·6	Yq11 21→Yq11.23
	Y-294	2·1	Yq11 21→Yq11.23
Y-specific repeated	Y-156	4·4	Yp
	Y-182	1·85	Yp
	Y-190	3·5	Yp
	Y-216a	2·4	Yq
	Y-219	5·1	Yp
	Y-223a	0·75	Yp
	Y-286	1·7	Hybridizes with 5·2 kb *Hin*dIII fragment on Yp and 1·7 kb fragment on Yq
Y-specific single copy plus non-Y single copy	Y-157a	3·7	Yq11 21→Yq11.23
	Y-198	1·2	Yq11.21→Yq11.23
	Y-221	2·4	Yq11.21→Yq11.23
	Y-227	2·8	Yp
	Y-228	2·75	Yp
	Y-280	2·2	Yp

in most XX males (Guellaen *et al.* 1984; Koenig, Moisan, Heilig & Mandel, 1985; Page, de la Chapelle & Weissenbach, 1985; Müller *et al.* 1986*a*; Müller, Lalande, Donlon & Latt, 1986*c*; Affara *et al.* 1986; Vergnaud *et al.* 1986). The percentages of Y-DNA-positive XX males reported so far range between 60% (Vergnaud *et al.* 1986) and 80% (Müller *et al.* 1986*a*,*c*; Müller, Latt & Donlon, 1987*b*). The failure to detect Y-DNA in all individuals studied thus far may be due to one of three reasons. First, individuals in whom no Y-DNA can be detected using any of the available probes could carry very small portions of the Y chromosome. The second possibility is that XX males who are Y-probe negative are chimaeras of the types 46,XX/46,XY or 46,XX/46,X,del(Y) or 46,XX/46,X,t(X;Y) and that the cells used for DNA extraction only contained the 46,XX cell line. This is consistent with earlier findings of such karyotypes in intersex partients and the high degree of variability in their tissue distribution frequencies (Müller *et al.* 1983). Third, some Y-probe negative 46,XX males may in fact be 46,XX true hermaphrodites that escaped clinical detection. Unlike 46,XX males, most 46,XX true hermaphrodites have been shown to be negative with any of the available Y probes (Vergnaud *et al.* 1986; Müller *et al.* 1986*c*; U. Wolf, personal communication).

Application of *in situ* hybridization to Y-DNA-positive XX males (Magenis *et al.* 1984*b*; Andersson, Page & de la Chapelle, 1986) and to a 47,XXX male (Müller *et al.* 1987*b*) demonstrated presence of the Y-DNA in these individuals on the tip of the short arm of one X chromosome. These findings further support the notion that an aberrant X–Y interchange during paternal meiosis is the most frequent underlying cause of the XX male syndrome.

Studying a total of 16 46,XX males and one 47,XXX male, we found Y-chromosomal DNA sequences in 13 of the XX males (i.e. 80%) and in the 47,XXX male (Müller *et al.* 1986*a*,*c*, 1987*b*; Table 2). The amount of Y-chromosomal DNA present in these male individuals varied considerably. As is shown in Table 2, probes Y-280 and Y-286 hybridized with DNA from all Y-DNA-positive individuals. Two 46,XX males were positive with only these two probes. Two other 46,XX males were positive with a total of four probes (Y-227; Y-228; Y-280; Y-286), the rest gave a positive hybridization signal with several additional probes.

The probes hybridizing to DNA from the XX males were assigned to the short arm of the Y chromosome in an XX male that carried a cytogenetically well-defined translocation of Yp to one of his X chromosomes (t(X;Y)(p22.33;p11.2); Magenis *et al.* 1982). All probes hybridizing with DNA of the cytogenetically normal 46,XX males also hybridized with DNA of the 46,XX male carrying the X;Y translocation. Conversely, all chromosome Y-specific DNA probes found not to hybridize with the DNA of any of our panel of XX males were negative in the DNA of the translocation patient as well. Probes Y-190 and Y-156, which hybridize to the DNA of 46,XX males,

Table 2. *Hybridization of Y-specific DNA probes with DNA from sixteen 46,XX males and a 47,XXX male*

	46,XX males																47,XXX
Probe	460*	462*	548*	756*	775*	693‡	547‡	#11†	(p22.33;p11.2)*	GM2626*	GM2670*	102*	510‡	GM1189*	481*	385‡	male‡
Y-190	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	+
Y-156	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	+
Y-182	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	+
Y-223a	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	+
Y-219	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	+
Y-227	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	+
Y-228	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	+
Y-280	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	+
Y-286	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	+
Y-198	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−
Y-253	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−
Y-202	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Y-216a	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Y-221	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Y-294	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Y-214	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Y-157a	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

Probes Y-198; Y-253; Y-202, Y-216a; Y-221; Y-294; Y-214; Y-157a were assigned to Yq11.21→Yq11.23 (Müller *et al.* 1987*b*) in patients with 46,X,del(Y)(pter→q11.23:) and 45,X/46,X,del(Y)(pter→q11.21:) karyotypes (see Müller *et al.* 1986*a*).

* From Müller *et al.* 1986*a*,*c*.

† From Müller *et al.* 1986*c*.

‡ From Muller *et al* 1987*b*

were also assigned to Yp by *in situ* hybridization (Muller *et al.* 1986*a*,*c*, 1987*a*).

46,XY females

Whilst most 46,XY females have a cytogenetically normal Y chromosome, a deletion of the Y short arm was detected in some individuals (Rosenfeld *et al.* 1979; Magenis *et al.* 1984*a*; Disteche *et al.* 1986). Unlike 'classic' Swyer's syndrome patients, however, these individuals had Turner stigmata in addition to gonadal dysgenesis. Several Y-chromosomal DNA probes were not detected by molecular hybridization in one of three 46,XY females that displayed no detectable Y-chromosomal aberration (Müller *et al.* 1986*c*).

Our failure to demonstrate absence of Y-chromosomal DNA sequences in the remaining two cases may be due either to the existence of deletions which are too small to be detected by our probes or to point mutations affecting the testis-determining gene(s). In addition, two 46,XY females with cytogenetically detectable deletions of Yp (Disteche *et al.* 1986) were found to be negative when tested with four of our Y probes (Müller *et al.* 1986*b*). Several cloned Y-chromosomal DNA sequences were also absent in a female patient with a dicentric Y chromosome (Müller *et al.* 1986*a*). The hybridization results obtained with the DNA of these patients are summarized in Table 3.

Deletion map of testis-determining locus on Yp

The findings obtained in the 46,XX males and in the 46,XY females allow the construction of a deletion map of Yp with respect to the testis-determining locus (Müller *et al.* 1986*a* and Fig. 1). Assuming that most of the Y-chromosomal translocations in XX males are contiguous, the hybridization data in the XX males tested allow the distinction of three groups. Group I is positive with all short-arm probes investigated and comprises the largest percentage of 46,XX males (nine patients) and the 47,XXX male. Group II (two patients) is positive with four of these probes and Group III (two patients) with only two of these. Since probes Y-156, Y-182, Y-190, Y-223a, and Y-219 also hybridize with DNA of the female patient with the dicentric Y chromosome, these probes must hybridize closest to the centromere. Probes Y-227 and Y-228 hybridize with DNA of the group II XX males and not with DNA of the dic(Y) female and thus detect sequences distal of those detected by the cluster of probes nearest to the centromere. This is further supported by hybridization of these probes with DNA of a male with a dicentric Y chromosome (unpublished data). Finally, probes Y-280 and Y-286 detect Y DNA in two additional XX males and hybridize with sequences most distal on Yp. Since these latter probes do not hybridize with any of the 46,XY females with Y-chromosomal deletions, they appear closest to the testis-determining locus. As yet, we do not know whether they are proximal or distal to this locus. As a result, our data, together with findings of others describing the pseudoautosomal region of Yp (Cooke, Brown & Rappold, 1985; Simmler *et al.* 1985; Goodfellow, Darling, Thomas & Goodfellow, 1986) suggest that the Y-chromosome short arm may be subdivided into five regions. These can be assigned the designations Yp I–Yp V, with Yp II being the testis-determining region. It should be stressed, however, that Yp II and Yp III might exchange their

Table 3. *Hybridization of eight Y-chromosomal DNA probes with DNA from five 46,XY females and a 46,X,dic(Y) female*

	46,XY females					
Probe	#740*	#620*	#651*	46,XYp−† #1	46,XYp−† #2	46,X,dic(Y)*
Y-156	+	+	red ‡	+	red ‡	+
Y-190	+	+	red.‡	+	n.t.	+
Y-227	+	+	−	−	−	−§
Y-228	+	+	−	−	−	−
Y-280	+	+	−	−	−	−
Y-286	+	+	−¶	−¶	−¶	−¶
Y-198	+	+	+	+	+	+
Y-253	+	+	+	+	+	+

* From Muller *et al.* (1986*a*,*c*).
† From Muller *et al.* (1986*b*) and unpublished
‡ Hybridization signal drastically reduced as compared to normal 46,XY male controls
§ Erroneously '+' in table 2 of Müller *et al.* (1986*a*)
¶ 5·2 kb Yp-specific *Hin*dIII fragment missing
n.t., not tested.

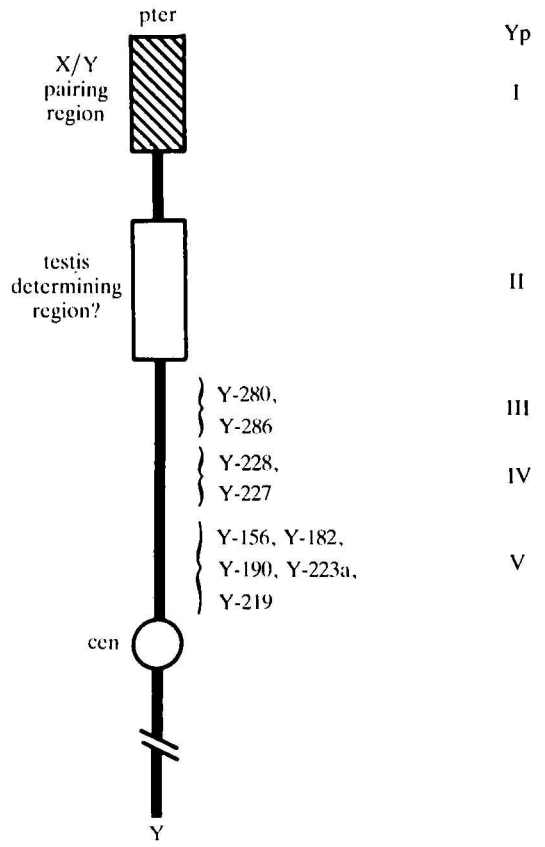

Fig. 1. Schematic diagram of the relative arrangement of Y-chromosomal DNA sequences hybridizing with various probes in relation to the testis-determining locus on Yp. Distances given in the map merely reflect the order of the probes and not physical or genetic distances of the probes from the testis-determining locus. For further details, see text.

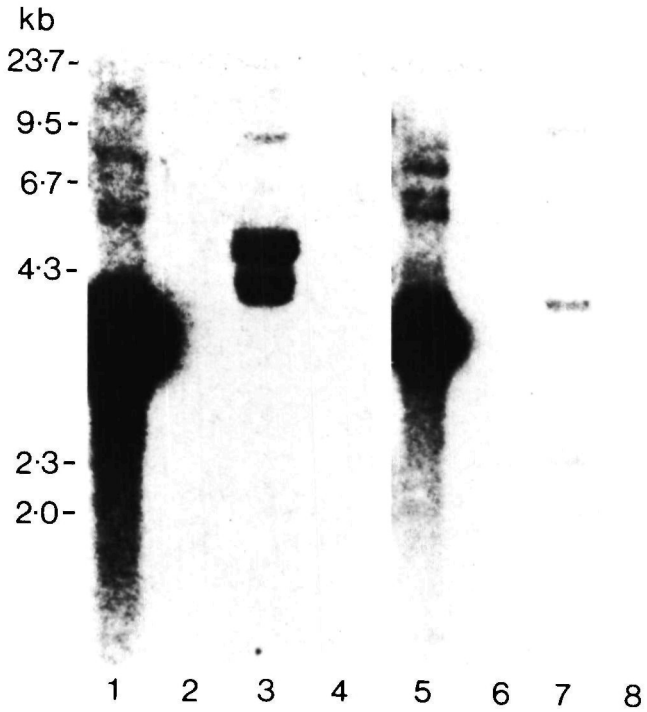

Fig. 2. Differential hybridization of probe Y-190 with male (lanes 1, 3, 5, 7) and female (lanes 2, 4, 6, 8), DNA from man (lanes 1, 2, 5, 6), orang-utan (lanes 3, 4), and rhesus monkey (lanes 7, 8). Hybridization conditions were as described in Müller *et al.* (1986*c*).

positions depending on whether the sequences hybridizing with Y-280 and Y-286 turn out to be proximal or distal to Yp II. Using different DNA probes and different patients, deletion maps similar to ours have been published by others (Vergnaud *et al.* 1986; Page *et al.* this symposium).

While regions Yp III–Yp IV are defined by probes that hybridize either with Y-specific single-copy DNA sequences or with a Y-specific single-copy sequence in addition to an autosomal sequence, all probes assigned to region Yp V were found to hybridize with repeated DNA (Müller *et al.* 1986*a*). It is tempting to speculate that the proximal region of Yp is primarily composed of Y-specific repeated DNA sequences and that Y-specific repeats become less abundant in more distal regions of Yp. The repeats of region Yp V do not appear to be related to Y-chromosomal centromeric alphoid satellite DNA (Wolfe *et al.* 1985). Unlike the centromeric repeats (Wolfe *et al.* 1985), the repeated DNA sequences of region Yp V are evolutionarily conserved in the great apes (DNA from orang-utan was tested) and, to a lesser degree, in rhesus monkeys (Fig. 2). Furthermore, the copy number of several of these repeats was found be be reduced in two XY females whose Y chromosome definitely had a centromere and the paracentromeric region of Yp (Müller *et al.* 1986*a*,*b*,*c*).

Chromosome walking on Yp and preliminary pulsed-field gel electrophoresis investigations

Given the putative close proximity of Y-286 and Y-280 to the testis-determining locus, we initiated chromosome-walking experiments (Fig. 3). Such experiments might eventually reveal Y-chromosomal DNA sequences that are evolutionarily conserved and therefore likely candidates for genes with important functions, e.g. testis differentiation. A partial Sau 3A digest library was constructed in vector EMBL 3 from DNA of a normal male (46,XY) individual according to Frischauf, Lehrach, Poustka & Murray (1983). The library was screened with probe Y-286 which detects two Y-chromosomal *Hin*dIII restriction fragments, one on Yp, the other on Yq (Müller *et al.* 1986*a*). Several phages were isolated and three were found to derive from Yp. Subcloning of the insert of one phage revealed a restriction fragment that hybridizes to a DNA sequence on Yp in addition to an autosomal sequence (Y-286/1) (Fig. 3). Using this

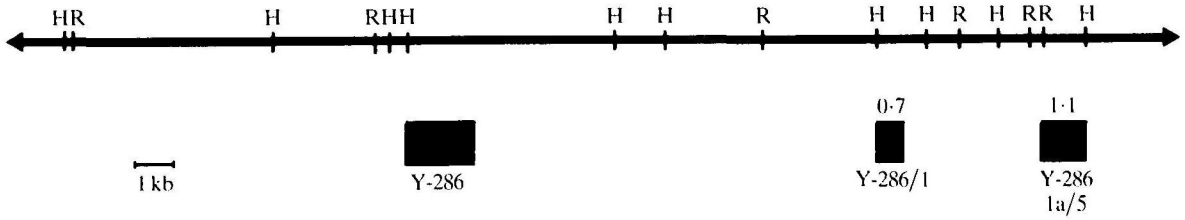

Fig. 3. Restriction map of the Y-286 region (H = *Hin*dIII, R = *Eco*RI). For details, see text.

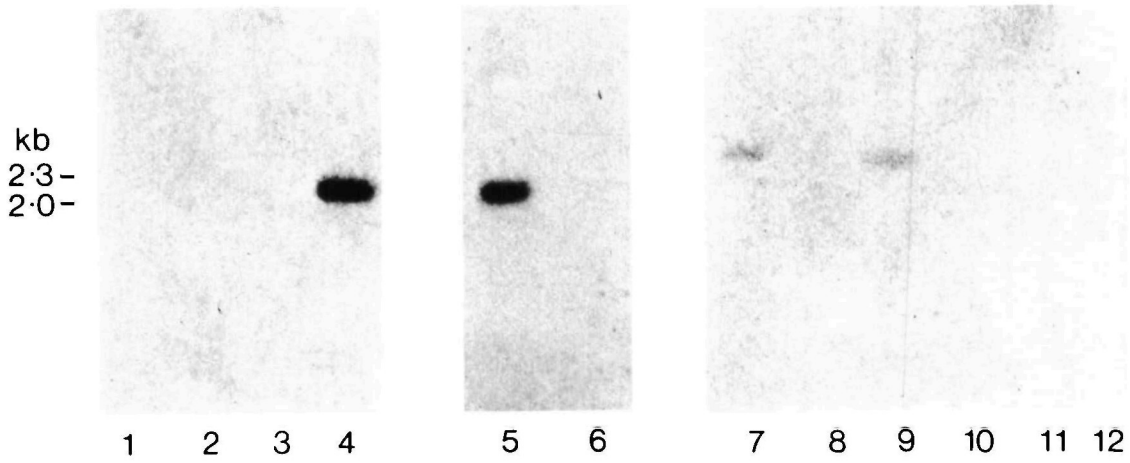

Fig. 4. Differential hybridization of probe Y-286/1a5 (see Fig. 2) with male (lanes 2,4,5,7,9,11) and female (lanes 1,3,6,8,10,12), DNA from horse (lanes 1,2), man (lanes 3,4), orang-utan (lanes 5,6), baboon (lanes 7,8), mandrill (lanes 9,10), mouse (lanes 11,12). Hybridization conditions were as described in Müller *et al.* (1986*c*).

fragment as a hybridization probe, an additional phage was isolated and some of the inserts subcloned were found to hybridize with single-copy sequences on Yp. These probes were used for studies of evolutionary conservation and revealed to be only moderately conserved under stringent hybridization conditions (for hybridization conditions, see Müller *et al.* 1986*c*). While DNA sequences hybridizing with these probes were found to be male-specific in the great apes (orang-utan) and Old World monkeys (baboon, mandrill, rhesus monkey), no hybridization could be detected in the one species of New World monkeys tested (tamarin) or in prosimians (lemurs and galagos), horses, bovines and rodents (mouse). An example of the hybridization pattern of one of these probes (Y-286/1a5; Fig. 3) with DNA of various primates is given in Fig. 4.

A long-range restriction map of the region surrounding the Y-286 DNA sequence has been initiated using field-inversion gel electrophoresis (U. Müller & M. Lalande, unpublished results). This technique allows the separation of DNA fragments in a size range of 10–2000 kb. These experiments should permit the determination of the proximity of probe Y-286 to *TDF* by comparing its hybridization pattern over large genetic map distances in normal males relative to XX males within the three groups described above.

In conclusion, hybridization of DNA from XX males and XY females with Y-specific DNA probes allowed the delineation of the putative testis-determining locus on Yp. Y-chromosomal DNA sequences detected by two probes (Y-280; Y-286) appear to be close to this locus. These probes are now being used for chromosome-walking experiments and pulsed-field gel electrophoresis studies that might eventually allow the isolation of the gene(s) required for normal testis differentiation.

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References

Affara, N. A., Ferguson-Smith, M. A., Tolmie, J., Kwok, K., Mitchell, M., Jamieson, D., Cooke, A. & Florentin, L. (1986). Variable transfer of Y-specific sequences in XX males. *Nucleic Acids Res.* **14**, 5375–5387.

Andersson, M., Page, D. C. & de la Chapelle, A. (1986). Chromosome Y-specific DNA is transferred to

the short arm of X chromosome in human XX males. *Science* **233**, 786–788.

BIGOZZI, V., SIMONI, G., MONTALI, E., DALPRA, L., ROSSELLA, F., PIAZZINI, M. & BORGHI, A. (1980). 47,XXX chromosome constitution in a male. *J. med. Genet.* **17**, 62–66.

BÜHLER, E. (1980). A synopsis of the human Y chromosome. *Hum. Genet.* **55**, 145–175.

CARLE, G. F., FRANK, M. & OLSON, M. V. (1986). Electrophoretic separation of large DNA molecules by periodic inversion of the electric field. *Science* **232**, 65–68.

CASPERSSON, T., LOMAKKA, G. & ZECH, L. (1971). The 24 fluorescence patterns of human metaphase chromosomes – distinguishing characters and variability. *Hereditas* **67**, 89–102.

COOKE, H. J., SCMIDTKE, J. & GOSDEN, J. R. (1982). Characterization of a human Y chromosome repeated sequence and related sequences in higher primates. *Chromosoma* **87**, 491–502.

COOKE, H. J., FANTES, J. & GREEN, D. (1983). Structure and evolution of human Y chromosome DNA. *Differentiation* **23 (Suppl.)**, S48–S55.

COOKE, H. J., BROWN, W. R. A. & RAPPOLD, G. A. (1985). Hypervariable telomeric sequences from the human sex chromosomes are pseudoautosomal. *Nature, Lond.* **317**, 687–692.

DE LA CHAPELLE, A. (1981). The etiology of maleness in XX men. *Hum. Genet.* **58**, 105–116.

DISTECHE, C. M., CASANOVA, M., SAAL, H., FRIEDMAN, C., SYBERT, V., GRAHAM, J., THULINE, H., PAGE, D. C. & FELLOUS, M. (1986). Small deletions of the short arm of the Y chromosome in 46,XY females. *Proc. natn. Acad. Sci. U.S.A.* **83**, 7841–7844.

FERGUSON-SMITH, M. A. (1966). X-Y chromosomal interchange in the aetiology of true hermaphroditism and of XX Klinefelter's syndrome. *Lancet* **ii**, 475–476.

FRISCHAUF, A. M., LEHRACH, H., POUSTKA, A. & MURRAY, N. (1983). Lambda replacement vectors carrying polylinker sequences. *J. molec. Biol.* **170**, 827–842.

GOODFELLOW, P. J., DARLING, S. M., THOMAS, N. S. & GOODFELLOW, P. N. (1986). A pseudoautosomal gene in man. *Science* **234**, 740–743.

GUELLAEN, G., CASANOVA, M., BISHOP, C., GELDWERTH, D., AUDRE, G., FELLOUS, M. & WEISSENBACH, J. (1984). Human XX males with Y single-copy DNA fragments. *Nature, Lond.* **307**, 172–173.

JOST, A., MAGRE, S. & AGELOPOULOU, A. (1981). Early stages of testicular differentiation in the rat. *Hum. Genet.* **58**, 59–63.

KOENIG, M., MOISAN, J. P., HEILIG, R. & MANDEL, J. L. (1985). Homologies between X and Y chromosomes detected by DNA probes: localization and evolution. *Nucleic Acids Res.* **13**, 5485–5501.

MADAN, K. & WALKER, S. (1974). Possible evidence for Xp+ in an XX male. *Lancet* **i**, 1223.

MAGENIS, R. E., WEBB, M. J., MCKEAN, R. S., TOMAR, D., ALLEN, L. J., KAMMER, H., VAN DYKE, D. L. & LOVRIEN, E. (1982). Translocation (X;Y) (p22.3; p11.2) in XX males: etiology of male phenotype. *Hum. Genet.* **62**, 271–276.

MAGENIS, R. E., TOCHEN, M. L., HOLAHAN, K. P., CAREY, T., ALLEN, L. & BROWN, M. G. (1984*a*). Turner syndrome resulting from partial deletion of Y chromosome short arm: localization of male determinants. *J. Pediatrics* **105**, 916–919.

MAGENIS, E., TOMAR, D., SHEEHY, R., FELLOUS, M., BISHOP, C. & CASANOVA, M. (1984*b*). Y short arm material translocated to distal X short arm in XX males: evidence from in situ hybridization of Y-specific single copy DNA probes. *Am. J. hum. Genet.* **36**, 102S.

MÜLLER, U., MAYEROVA, A., DEBUS, B., FRACCARO, M., GILGENKRANTZ, S., GLATZL, J., MADAN, K., PFEIFFER, R. A., PRADER, A. & ZUFFARDI, O. (1983). Correlation between testicular tissue and H-Y phenotype in intersex patients. *Clin. Genet.* **23**, 49–57.

MÜLLER, U., DONLON, T., SCHMID, M., FITCH, N., RICHER, C. L., LALANDE, M. & LATT, S. A. (1986*a*). Deletion mapping of the testis determining locus with DNA probes in 46,XX males and in 46,XY and 46,X,dic(Y) females. *Nucleic Acids Res.* **14**, 6489–6505.

MÜLLER, U., LALANDE, M., DISTECHE, C. M. & LATT, S. A. (1986*b*). Construction, analysis, and application to 46,XY gonadal dysgenesis of a recombinant phage DNA library from flow-sorted human Y chromosomes. *Cytometry* **7**, 418–424.

MÜLLER, U., LALANDE, M., DONLON, T. & LATT, S. A. (1986*c*). Moderately repeated DNA sequences specific for the short arm of the human Y chromosome are present in XX males and reduced in copy number in an XY female. *Nucleic Acids Res.* **14**, 1325–1340.

MÜLLER, U., DONLON, T. A., KUNKEL, S. M., LALANDE, M. & LATT, S. A. (1987*a*). Y-190, a DNA probe for the sensitive detection of Y-derived marker chromosomes and mosaicism. *Hum. Genet.* **75**, 109–113.

MÜLLER, U., LATT, S. A. & DONLON, T. (1987*b*). Y-specific DNA sequences in male patients with 46,XX and 47,XXX karyotypes. *Am. J. med. Genet.* (in press).

PAGE, D. C., DE LA CHAPELLE, A. & WEISSENBACH, J. (1985). Chromosome Y-specific DNA in related human XX males. *Nature, Lond.* **315**, 224–226.

ROSENFELD, R. G., LUZZATTI, L., HINTZ, R. L., MILLER, O. J., KOO, G. C. & WACHTEL, S. S. (1979). Sexual and somatic determinants of the human Y chromosome. Studies in a 46,XYp– phenotypic female. *Am. J. hum. Genet.* **31**, 458–468.

SCHEMPP, W. & MÜLLER, U. (1982). High resolution replication patterns of the human Y chromosome. Intra- and interindividual variation. *Chromosoma* **86**, 229–237.

SCHWARTZ, D. C. & CANTOR, C. R. (1984). Separation of yeast chromosome-sized DNAs by pulsed field gel electrophoresis. *Cell* **37**, 67–75.

SIMMLER, M. C., ROUYER, F., VERGNAUD, G., NYSTROM-LAHTI, M., NGO, K. Y., DE LA CHAPELLE, A. & WEISSENBACH, J. (1985). Pseudoautosomal DNA sequences in the pairing region of the human sex chromosomes. *Nature, Lond.* **317**, 692–697.

Simpson, J. L., Blagowidow, N. & Martin, A. O. (1981). XY gonadal dysgenesis: genetic heterogeneity based upon clinical observations, H-Y antigen status and segregation analysis. *Hum. Genet.* **58**, 91–97.

Swyer, G. I. M. (1955). Male pseudohermaphroditism: a hitherto undescribed form. *Br. Med. J.* **2**, 709–712.

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 chromsome based on DNA hybridization. *Am. J. hum. Genet.* **38**, 109–124.

Verma, R. S., Dosik, H., Scharf, T. & Lubs, H. A. (1978). Length heteromorphisms of fluorescent (f) and non-fluorescent (nf) segments of human Y chromosome: classification, frequencies, and incidence in normal Caucasians. *J. med. Genet.* **15**, 277–281.

Wachtel, S. S., Koo, G. C., Breg, W. R., Thaler, H. T., Dillard, G. M., Rosenthal, I. M., Dosik, H., Gerald, P. S., Salenger, P., New, M., Lieber, E. & Miller, O. J. (1976). Serologic detection of a Y-linked gene in XX males and XX true hermaphrodites. *New Engl. J. Med.* **295**, 750–754.

Wolfe, J., Darling, S. M., Erickson, R. P., Craig, I. W., Buckle, V. J., Rigby, P. W. J., Willard, H. F. & Goodfellow, P. N. (1985). Isolation and characterization of an alphoid centromeric repeat family from the human Y chromosome. *J. molec. Biol.* **182**, 477–485.