Localisation of Y chromosome sequences in normal and 'XX' males

V J BUCKLE*, Y BOYD*, N FRASER*, P N GOODFELLOW†, P J GOODFELLOW†, J WOLFE‡, AND I W CRAIG*

From *the Genetics Laboratory, Biochemistry Department, Oxford University, South Parks Road, Oxford OX1 3QU; †Human Molecular Genetics Laboratory, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX; and ‡MRC Human Biochemical Genetics Unit, The Galton Laboratory, 4 Stephenson Way, London NW1 2HE.

SUMMARY Three unique sequences derived from the Y chromosome have been mapped within the human genome. A Y specific sequence *DYS20* is localised to Yq11·2. *DXYS25* and *DXYS27* are both X-Y homologous sequences which map to the Y short arm and to Xq21. *DXYS25* maps more distally than *DXYS27*, on the Y short arm and on the X long arm. Y specific restriction fragments for these two sequences are shown to be present in the genome of two XX males, and an aberrant signal for *DXYS25* is demonstrated at the tip of an X chromosome short arm in one XX male by in situ hybridisation. The implications of these findings for the location of the testis determining factor are discussed.

Molecular homology between the sex chromosomes in man is maintained in the pseudoautosomal region by recombination.¹ Other sequences isolated from the short and the long arm of the Y chromosome also show homology with the X,²⁻⁴ but such homologies are not maintained by recombination and may result from recent transpositions of material to the Y chromosome.⁵

From analysis of Y chromosome rearrangements and their associated phenotypes, the testis determinant (*TDF*) has been assigned to the proximal Y short arm⁶ although cytogenetic observations in some XX males suggest a more distal location.⁷ Cloned sequences with Y specific restriction fragments have been shown to be present in the genome of XX males.⁸ This is presumed to reflect an abnormal recombination event between X and Y chromosomes in the paternal meiosis, resulting in transfer of Y chromosomal material, including the testis determining gene, onto the paternal X chromosome.⁹ XX males appear to be heterogeneous in terms of the amount of Y material present, and in one third of cases studied no Y specific restriction fragments have been observed.¹⁰ These cases may result from transfer of relatively little Y chromosome material, including the *TDF* but none of the Y specific fragments so far ex-

amined, or may be due to undetected mosaicism involving the gonadal tissues, or to mutations elsewhere in the genome.¹¹ If we assume that XX males have acquired the *TDF* through a simple misplaced X/Y recombination site, then the *TDF* must be located distal to the majority, and possibly all, of the Y sequences identified to date which are transferred in XX males (other than the pseudoautosomal sequences). However, an aetiology for XX males due to more complicated internal recombinational events cannot be excluded.¹⁰ Clear physical assignments for sequences on the Y chromosome may be informative in this respect. We report here the localisation of three single copy sequences derived from the human Y chromosome. The sequences were mapped by in situ hybridisation and by somatic cell hybrid studies, on normal and rearranged sex chromosomes and in XX males.

Materials and methods

MOLECULAR PROBES

Single copy human sequences have been isolated from a cosmid library made with DNA from the 3E7 human/mouse hybrid retaining only Y chromosomal human material.¹²

DXYS25. Sequences p75/78 and p75/79 are different subclones of cosmid 75.¹³ They have been previously mapped to the Y chromosome and to

Xq13→qter, and are presumed to recognise the same segment of X-Y homologous sequence. p75/79 recognises an X specific *Eco*RI restriction fragment of 4.9 kb and a Y specific fragment of 2 kb. DXYS27. Sequence p59 γ is a 1.4 kb insert cloned into pUC9 and is also X-Y homologous (Fraser *et al.* in preparation). p59 γ recognises X specific restriction fragments of 3 kb (*Pst*I) and 4 kb (*Bam*HI) and Y specific restriction fragments of 2.1 kb (*Pst*I) and 1.8 kb (*Bam*HI). DYS20. Sequence p69/6 is Y specific and maps to the heterochromatic part of the Y chromosome by hybrid analysis.^{1,2}

CELL LINES AND HYBRIDS

Chromosome preparations were obtained from normal male lymphocytes, from TEL26, a lymphoblastoid cell line with an interstitial deletion of the X chromosome long arm, 46,Y, del(X)(q13.3→q21.3), provided by Professor P Pearson, and from GIM and WER which are lymphoblastoid cell lines derived from 46,XX males, provided by Dr M Fellous (reference 8, cases 1 and 3 respectively).

DNA was prepared from a variety of cell lines and hybrids, using standard techniques. The sex chromosome constitution and relevant references

for these lines are given in the table. The cell line GM1416 was obtained from the Human Genetic Mutant Cell Repository. DNA from the hybrids H10, H11, H33, and their corresponding revertants (selected for resistance to 6-thioguanine) were a gift from Professor B Migeon. The X chromosome

TABLE Localisation of DXYS25 and DXYS27 by hybridisation of probes p75/79 and p59 γ to DNA prepared from a range of human and hybrid cell lines.

DNA	Human sex chromosome content	DXYS25	DXYS27
46,XX	X	+	+
46,XY	X and Y	+	+
MOG 13/9 ¹⁵	X	+	+
MOG revertant	None	-	-
GM1416 (48,XXXX)	X dosage	+	+
OXEN (49,XYYY) ¹⁶	Y dosage	+	+
HORL9 11R8B ¹⁷	Xpter→qter	+	+
MCP6 ¹⁸	Xq13→qter	+	+
TEL26	del(X)(q13.3→q21.3)Y	-X band- +Y band+	-
H10 ¹⁹	Xq13.1→qter	+	+
H10 revertant	None	-	-
H33	Xq22→qter	-	-
H33 revertant	None	-	-
H11 ²⁰	Xq26→qter	NT	-
H11 revertant	None	NT	-

FIG 1 Grain distribution over the sex chromosomes in 36 normal male cells and in 31 TEL26 cells probed with p75/79. Scores for the Y chromosomes are combined.

breakpoint in the parental human cell line to hybrid H11 has been confirmed as Xq26 (GM3552 Human Genetic Mutant Cell Repository). Filters were hybridised to a range of probes derived from the X chromosome in order to confirm the representation of X chromosome material in the hybrids.

HYBRIDISATION TECHNIQUES

In situ hybridisation techniques have been described in detail elsewhere.¹⁴ Probes were labelled with I¹²⁵ dCTP (Amersham Int) to specific activities of 1 to 3 $\times 10^8$ dpm/ μ g and after hybridisation the slides were exposed for two to six days. Filter hybridisations were performed by standard techniques. Probes were nick translated to a specific activity of 10⁸ dpm/ μ g and filters were washed to stringencies of 0.2 to 0.5 $\times$ SSC at 63° to 68°C.

Results

LOCALISATION OF DXYS25, DXYS27, AND DYS20 IN NORMAL MALES

Probes p75/78 and p59 γ both hybridised to the short

arm of the Y chromosome and to the X chromosome at q21, while probe p69/6 hybridised only to the Y long arm.

In the analysis of 25 chromosome spreads from a normal male which had been hybridised in situ with p75/78, 39 grains (11.6% of all grains scored) were on the X or Y chromosome (including 30% of all grain clusters). Thirty-six cells were examined for the location of grains on the X and Y chromosomes alone. Of 26 grains scored on the Y chromosome, 20 (77%) lay in bands p11.2 $\rightarrow$ pter. Seventy percent of the grains in this region were in the distal band Yp11.3. Sixteen (53%) of the 30 grains scored (including all of the seven grain clusters observed) for the X chromosome were in band Xq21, with the majority of these in the distal half of that band (fig 1).

Analysis of the in situ hybridisation of p59 γ in 24 normal male cells indicated a broadly similar grain distribution to that observed for p75/78. However, the distribution of grains was more proximal on both the Y short arm and the X long arm (fig 2). Ten of

FIG 2 Grain distribution over the sex chromosomes in 24 normal male cells and in 27 TEL26 cells, probed with p59 γ . Scores for the Y chromosomes are combined.

the 11 grains scored on the Y chromosome, including all three clusters observed, were on the short arm, and the heaviest signal was not observed in the distal band p11.3 as it was for p75/78. Ten of the 20 grains on the X were over band q21, and eight, including all clusters scored on the X, were in the proximal region of that band.

In situ hybridisation of both probes to the lymphoblastoid cell line TEL26 provided confirmation of their localisations on the Y chromosome, and grain counts for that chromosome from normal males and TEL26 have been combined in figs 1 and 2. For p75/78, 50% of all grains scored on the Y chromosome in 67 cells lay in band p11.3, while for p59 γ , most grains (39%) in 51 cells lay in band p11.2. Furthermore, because no signal comparable to that observed for the Y chromosome in TEL26

was detected on the deleted X long arm, it was concluded that the deletion has removed both DXYS25 and DXYS27, thereby confirming their assignment to Xq21.

Independent evidence for the assignment of DXYS25 and DXYS27 to the X and Y chromosomes, and for their localisation on the X chromosome to Xq21, was obtained by hybridisation to restriction enzyme digests of DNA from a variety of human cell lines and somatic cell hybrids (table, fig 3). When appropriate restriction digests of TEL26 (46,Y,del(X)(q13.3→q21.3)) were probed with p75/79 and p59 γ , no X specific band was seen, thereby localising these sequences to the region of X chromosome removed by the deletion.

When p69/6 was hybridised in situ to normal male chromosomes, 8% (14 grains) of all grains scored in 10 cells were localised on the Y chromosome. This represents 16% of grains scored per haploid genome, of which the Y chromosome represents 2% in length. The Y was examined for regional distribution of grains in 20 cells. Eighteen (82%) of the 22 grains scored were localised in the euchromatic part of the long arm, Yq11.2 (fig 4).

LOCALISATION OF DXYS25 AND DXYS27 IN XX MALES

Both X and Y specific restriction fragments for p75/79 and p59 γ were found in restriction digests of lymphoblastoid cell line DNA prepared from two 46,XX males (GIM and WER; figs 3 and 5). This indicates the presence of Y chromosomal DNA within the genome of the XX males. The location of DXYS25 in the cell line GIM was examined by in situ hybridisation with p75/78. Analysis of the grain

FIG 3 Hybridisation of p75/79 to *Eco*RI digested DNA, showing an X specific band of 4.9 kb and a Y specific band of 2 kb (see table). (a) Localisation of DXYS25 to the TEL26 deletion Xq13.3→q21.3. Lane 1, 46,XX; lane 2, TEL26; lane 3, OXEN (49,XYYYY). *Bam*HI digested DNA probed with p59 γ gave a similar localisation for DXYS27 (data not shown, see table). (b) Demonstration of the presence of Y specific restriction fragments for p75/79 in the XX male cell lines WER and GIM. Lane 1, GM1416 (48,XXXX); lane 2, WER; lane 3, GIM; lane 4, OXEN.

FIG 4 Grain distribution over the Y chromosome in 20 normal male cells probed with p69/6.

FIG 5 Demonstration of the presence of Y specific fragments in the XX male cell lines GIM and WER, when probed with p59 γ . (a) *Pst*I digested DNAs showing an X specific band of 3 kb and a Y specific band of 2.1 kb. Lane 1, 46,XX; lane 2, GIM; lane 3, 46,XY. (b) *Bam*HI digested DNA showing an X specific band of 4 kb and a Y specific band of 1.8 kb. Lane 1, 46,XX; lane 2, WER; lane 3, 46,XY; lane 4, *Hind*III digested DNA.

distribution on both of the X chromosomes in 23 cells indicated the existence of two hybridisation sites (fig 6). Fifteen of the 23 grains scored on the X long arm were localised in band q21, which is the same position as the localisation for *DXYS25* on the X chromosome of normal males. Another signal, however, was observed at the tip of the X short arm, at about half the intensity of that observed for Xq21. This suggests that only one of the two X chromosomes in each cell carried the aberrant signal.

Discussion

DYS20 is a Y specific unique sequence which maps to the euchromatic part of the long arm by in situ hybridisation with probe p69/6. Another Y specific sequence,²¹ an X-Y homologous sequence,³ and an actin pseudogene with homologies throughout the genome²² have also been assigned to this region of the Y chromosome long arm. Wolfe *et al*¹² originally assigned *DYS20* and another similarly derived

sequence, *DYS21*, to the heterochromatic region of the long arm by deletion analysis using the AMIR hybrids.²³ These hybrids were thought to carry a Y chromosome breakpoint at the border of heterochromatin and euchromatin on the long arm, and both *DYS20* and *DYS21* mapped distal to this breakpoint. The localisation of *DYS20* to Yq11.2 by in situ hybridisation indicates that the AMIR breakpoint is more proximal on the Y chromosome long arm than originally defined.

The X-Y homologous sequences *DXYS25* and *DXYS27* (recognised by probes p75/78 and p59 γ respectively) both map to the short arm of the Y chromosome, Yp11, and to band q21 on the X chromosome long arm. However, their precise localisation within these bands may differ since *DXYS25* appears to map more distally, by in situ hybridisation, in each case. The Y short arm is small (1 to 2 $\times 10^4$ kb of DNA) and difficult to subdivide accurately by cytogenetic analysis. However, when p75/78 was hybridised to the chromosomes of two normal males and TEL26, most grains were consis-

FIG 6 Grain distribution over the X chromosomes in 23 cells from the XX male cell line GIM when probed with p75/78.

tently scored at the distal tip of the Y short arm. After hybridisation of the same cell lines with p59y, in all three cases most grains were scored in the middle of the Y short arm.

The Y specific restriction fragment for DXYS25 is shown to be present in the genome of the XX male 'GIM', and the in situ hybridisation pattern of p75/78 to the X chromosomes of GIM provides clear evidence of transfer of the Y material to the tip of one X chromosome. This is consistent with the X-Y interchange model for the origin of XX males, first proposed by Ferguson-Smith.⁹

Vergnaud *et al.*¹⁰ have constructed a deletion map of the Y chromosome, comprising seven intervals, based on the presence/absence of Y specific restriction fragments in subjects with sex chromosome anomalies. The XX male cell lines GIM and WER contain two Y specific restriction fragments detected by the probe 47c,⁸ which have been assigned to intervals 1 and 2 on the Y short arm, according to the Vergnaud *et al.*¹⁰ classification. GIM (but not WER) also contains Y specific restriction fragments assigned to interval 3. Since WER contains Y specific restriction fragments for both DXYS25 and DXYS27, these two sequences can be assigned to intervals 1 to 2 of the Vergnaud classification. The fact that a difference can be detected in the localisation of DXYS25 and DXYS27 on the Y short arm by in situ hybridisation suggests that intervals 1 to 2 may consist of several thousand kilobases of DNA sequence.

The pseudoautosomal region has been estimated to comprise at most 5000 kb of DNA sequence.¹ The TDF, which maps to the Vergnaud interval 1, must reside below this region to preserve its functional and genetic identity, as must the X-Y homologous region represented by DXYS25. The X-Y homologous gene MIC2 maps to the tip of the Y chromosome²⁴ and its Y localisation cannot be distinguished from that of DXYS25 by in situ hybridisation. If the aetiology of all XX males results from the simple contiguous transfer to an X chromosome of a Y segment including the Y terminus, the order of sequences on the Y short arm would be: Ypter-pseudoautosomal region-MIC2-TDF-DXYS25. TDF is placed distal to DXYS25 because not all XX males receive the latter sequence as a result of the presumptive exchange (manuscript in preparation). Under the same premise of a simple transfer, the localisation of DXYS25 to the distal Y short arm would indicate that the testis determining factor is situated more distally than previously reported.⁶ This would be in agreement with the cytogenetic evaluation of Magenis *et al.*⁷ Further in situ localisation of sequences on the short arm of the Y chromosome, in combination with a deletion

map, would confirm the order of the Vergnaud intervals and the position of TDF.

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Correspondence and requests for reprints to Dr V Buckle, Genetics Laboratory, Department of Biochemistry, South Parks Road, Oxford OX1 3QU.