A long range restriction map of the pseudoautosomal region by partial digest PFGE analysis from the telomere

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ABSTRACT

The analysis of partial digestion products extending from the telomere of the human X and Y chromosomes, visualised by hybridisation to a probe located close to the telomere, was used to establish a restriction map of the pseudoautosomal region. In this highly polymorphic region with a 10-fold elevated recombination frequency in males we identified site or methylation differences between 7 different male and female cell lines and tissues, and derived an estimate of the size of the pseudoautosomal region of approximately 3 Megabases by comparing X and Y chromosomes. This size correlates well with previous estimates based on genetic arguments and argues against a strongly enhanced rate of exchange near telomeres in general. We identified a CpG rich and hypomethylated region within 500 kbp from the telomere, which might reflect structural features of mammalian telomeres, and a small number of (additional) CpG islands, which might represent candidate genes for the Turner phenotype in XO females.

INTRODUCTION

The observation of pairing between the human chromosomes X and Y (1, 2) somatic effects in 45,XO Turner females (3) and lack of inactivation of loci like STS and XG (4, 5) led to the proposal that X and Y carry a homologous segment of DNA at Xpter and Ypter. Sequences within this region can exchange by recombination and in consequence fail to show strict sex-linked inheritance. This pattern of inheritance has been described as pseudoautosomal (6). Several pseudoautosomal loci have been mapped to this region and exhibit from none, to partial sex linkage (7, 8, 9). The segregation patterns of these loci indicate that X/Y recombination results from a single obligatory meiotic crossing-over in the pseudoautosomal region (9).
It has also been shown that the pseudoautosomal region is a region of enhanced recombination in male meiosis since recombination occurs about 10 times more frequently than in female meiosis (10). This elevated recombination frequency provides an interesting opportunity to correlate genetic and physical distances.

So far there is no direct evidence on the size of the pseudoautosomal region. It has however been suggested that this region is quite small. From the approximately 5% recombination frequency in female meiosis, the frequency of isolation of pseudoautosomal clones and from the size of cytologically detectable deletions of this region a maximum size of 5 Mbp has been estimated (10, 11), well within the potential resolution range of PFGE (12, 13).

To attempt to determine the size and to derive information on the structure and methylation status of this highly polymorphic region, we have used pulsed field gradient gel techniques to derive physical maps of the telomeric regions of Xp and Yp in a number of different tissues and cell lines. By comparison of the physical maps between the X and Y chromosomes we should be able to map the proximal limits of the pseudoautosomal region, since restriction fragments extending beyond the border between pseudoautosomal and chromosome specific regions should differ in size.

The establishment of such long range restriction maps by PFGE usually relies on the analysis of single and double enzyme digests (14,15). Since many rare cutting site are clustered in CpG island sequences (16), each probe contributes mapping information over regions covering a few CpG islands. Especially in regions with a high density of those islands, in which therefore the average size of DNA fragments generated by enzymes cutting rarely in the mammalian genome ("rare cutters") is quite small, the establishment of long maps requires a number of closely spaced probes. This is not necessarily the case for approaches in which partial products are analysed, allowing the derivation of mapping information at longer distances from the probe. An especially powerful version of restriction mapping by partial digestion is the analysis of partial digestion products extending from either a natural DNA end or a completely cleaved restriction site, visualized by indirect end labelling (12).

The pseudoautosomal region of the short arm of the X and Y chromosomes is especially suitable for this purpose since a telomeric
probe is available (7). Here we exploit the structure of the pseudoautosomal regions to map from this naturally occurring DNA end.

MATERIALS AND METHODS

Cell lines and tissues

Restriction mapping by partial digests was performed in DNA from different male and female human lymphoblastoid cell lines (mostly from related individuals; unpublished) including the cell line GM 1416B (NIGMS Human Genetic Cell Repository, Camden, New Jersey) which contains 4 X-chromosomes (here called 4X); in the 853 cell line (also called 7631, 17) a CHO/human somatic cell hybrid containing only a human Y chromosome (here called 853(Y)); in the 578 cell line (18), a CHO/human somatic cell hybrid containing only a human X chromosome (here called 578 (X)); in sperm; and in male and female blood of unrelated individuals.

DNA preparation, PFGE, hybridisation

Conditions for DNA preparations in agarose blocks, pulsed field gel electrophoresis, DNA transfer, hybridisation conditions and filter regeneration have been described (19). For DNA markers on the PFGE we used wild type lambda clear multimers (x48.5 Kbp), Saccharomyces cerevisiae yeast markers (200, 320, 420, 550, 600, 700, 750, 800, 850, 950, 1000, 1200, 1250, 1400 Kbp's) Schizosaccaromyces pombe markers (3, 5, 7 Mb) (13 and personal communication) and Hansenula wingei, the chromosomes of which lie in the range of 500 Kbp and <3000 Kbp. A millimeter-grid copied on a transparent plastic foil was laid over the gel when taking a photograph, to simplify the evaluation of DNA fragment sizes relative to the marker sizes. Markers were usually run at both sides and sometimes in addition in the middle of the gel. Resolution of DNA fragments in the size range 0-600 Kbp was obtained by electrophoresis for 40h at 9V/cm, 92mA at 13°C using 40 second pulse times; in the 0-1500 Kbp size range by electrophoresis for 40h at 9.5V/cm, 95mA at 13°C using 100 second pulse times; in the 0-6Mbp size range by electrophoresis for 7 days at 2.2V/cm, 70mA at 13°C using 30-40 min pulse times. Electrophoresis was carried out in either hexagonal (1-6Mb) or square box ((0-1500Kbp) OFAGE gel systems, with the running conditions adapted from 12, 13 and 20. For some of the gels a combination of both "long" (2.2V/cm, 30-40 min pulse time, 6 days) and "short" (100 sec pulse time, 1-2 days) gel conditions were applied by changing the switching intervals.
after 6 days of gel run and continuing it on the shorter switching intervals for 1 or 2 more days. This resulted in a better resolution up to 1500 Kbp.

**Restriction enzymes and partial digestion conditions**

Restriction enzymes were purchased from NEB and were used as recommended by the manufacturers in complete digest reactions. Partial digests were carried out for 4h at 37°C (for NotI) and 50°C (for BssHII) with reduced amounts of restriction enzymes (e.g. 5,2,1,0.75,0.5,0.25,0.1 units) in a digestion mix of 200μl. The reaction was stopped by the addition of 0.2 mg of Proteinase K and 50mM EDTA for 30min at 50°C and the blocks were loaded onto gels directly after digestion. We routinely used 0.5-1 million cells per agarose block since a lower cell concentration tended to give better partial products, despite the slight reduction in signal.

**Probes**

The 'telomeric probe' used was 29A24, a HindIII fragment of 2.5 Kbp in pUC9, derived from the minisatellite of the DXYS20 locus (21). 29A24 is a repeated sequence, with 6-15 copies located in a single cluster approximately 25Kbp from the X and Y chromosome short arm telomeres, derived from the original cosmid CY29 at the proximal end (7). Probe 19b is a 0.9Kbp EcoRI fragment in pUC8 obtained from the MIC2 gene (9). MIC2 comprises the most proximal pseudoautosomal marker located less than 200 Kbp from the sex chromosome specific regions of X and Y (22 and own results). Probes were isolated from low melting point agarose and labelled by priming with random oligomers (23).

**RESULTS**

1. Distribution and differential methylation of CpG's in the first 500 Kbp of the X-Y short arm Telomere region

   a. Restriction mapping by complete digestion

Hybridisation with probe 29A24, approximately 25 Kbp from the telomere, to several PFGE filters with complete digests of 853 (see Materials and Methods) and male blood DNA with rare cutter enzymes such as NaeI, SacII, NotI, SalI, BssHII, SfiI, NruI, MluI, resulted in small digestion products (see Figure 1) in the size range of 100-250 Kbp. Several enzymes, such as MluI, SalI and NaeI gave "natural" partial products (see Fig. 1). Additionally, faint bands resulting from sites which are cut very partially due to differential methylation (sites shown in brackets), are
Long range restriction map at the telomere of the Y chromosome. PFGE analysis of 853(Y) genomic DNA digested with enzymes BssHII, SfiI, Sall, NotI/NruI double digest, NotI, NaeI and MluI. *Saccharomyces cerevisiae* chromosomes provided the markers which were separated at both sides of the gel. Sizes are shown in Kbp and the resultant long range restriction map is illustrated; partially cleaved sites are shown in brackets. Conditions of gel run see Material and Methods.

visible on a longer exposure of this autoradiogram. This observation of small digestion products gave us the first hint of a hypomethylated region with short distances between restriction endonuclease sites.
**Figure 2**

a. PFGE analysis of genomic DNA from different cell lines and tissues: sperm, 853, ('Y only'), GM 1416B (4X), male blood, female blood (from left to right) NotI enzyme amounts used were 2u and 0.5u run in parallel tracks. The 0.5u enzyme track produced partial fragments which all run in the limiting mobility (LM) range above 800 Kbp.

b. PFGE analysis of genomic DNA of the 4X lymphoblastoid cell line. Shown are 3 tracks where DNA has been digested with 5, 2 and 0.5 units NotI (from left to right) in a 4 hours digestion. Highly methylated sites are very weakly cut and thus can only be seen after prolonged exposure.

c. PFGE analysis of genomic DNA of sperm. Enzyme concentrations were 1, 2, 5, 20 units NotI (from left to right)
Diagram 1

A. Illustration of 7 different restriction maps derived by NotI partials of the tissues 4X, 853, sperm, male and female blood and male and female lymphoblastoid cell lines. The position of the sites with respect to their distance from the telomere is shown in kbp below. The vertical lines represent the restriction sites of NotI, the smaller vertical lines symbolizes the highly methylated, weakly cut sites. The number in brackets refer to the number of NotI sites (well-cut and partially cut) within the first 1500 Kbp of the corresponding tissue.

B. Illustration of 2 restriction maps of 853(Y) and 578(X), around MIC 2 with NotI.

C. Schematic diagram showing the BssH II cleaved sites in the 578 (X) and 853(Y) cell lines, summarizing the data of Fig 3abc, Fig 5a, b and of 853(Y) in the lower resolution range.

b. Restriction mapping by partial digestion with NotI

Due to the elevated recombination frequency and the highly polymorphic character of the human pseudoautosomal region mapping of
methylation or site specific differences is of particular interest. In order to
determine a map based on partial digestion 5 different rare cutter
enzymes were tested and NotI and BssHII were found to give especially
informative complete and partial restriction digests. We obtained 7 NotI
maps from tissues and cell lines of unrelated individuals, each spanning
about 1500 Kbp, using DNA from male and female blood, different male
and female lymphoblastoid cell lines, sperm and a cell line containing a
human Y chromosome on hamster background (see Material and
Methods). Due to the high frequency of NotI sites around the telomere, a
comparison of multiple gels giving optimal resolution over different
molecular weight ranges was necessary.

A selection of 3 gels is shown here (Fig. 2a, b, c), demonstrating most
of the sites summarized in Diagram 1. The human cell line GM 1416B with
4X chromosomes has 9 NotI cleavable sites within 1500 Kbp, with a
clustering of 7 NotI sites within the first 500 Kbp from the telomere
(Figure 2b). Within the 4X cell line the cleavage by NotI shows some
degree of heterogeneity with at least 3 very weakly cleaved NotI sites at
positions 90, 300 and 490 Kbp, which seem to be highly, but not
completely methylated and can only be seen after prolonged exposure.
The male and female lymphoblastoid cell lines from normal individuals
showed a clustering of sites at the same positions, with the exception of a
loss of cleavable sites at position 300 and 490 and an additional site at
position 350. Therefore, to confirm the data and to investigate the
differential methylation of sites and/or RFLP's, we also analysed the
region in male and female blood DNA samples (see Fig. 2a), producing the
same NotI sites as with the male/female lymphoblastoid cell line (Diagram
1, A). Male and female blood exhibit 6 cleaved NotI sites at the same
positions in the first 500 Kbp of the telomere, with an additional cleaved
NotI site at position 360 in both tissues, plus a cleaved NotI site only in
female blood of this individual at position 420. Sperm , a more
homogenous tissue and the cell line 853(Y) exhibit the smallest number
of cleaved NotI sites of all 7 tissues tested, with 4 sites in sperm (and 3 in
853) respectively within 1500 Kbp and a cluster of 3 sites in sperm (and 2
in 853) within the first 500 Kbp (Fig 2c, Diagram1,A). Therefore,
approximately half of the NotI sites present in blood and the
lymphoblastoid cell lines are methylated (or not present) in sperm and
853 DNA. Besides methylation differences in different tissues and cell
lines, there are also strong differences of band intensity of the partial products in each DNA due to weak or strong methylation.

c. Restriction mapping by partial digestion with BssHII

Partial digestion with BssHII was investigated to determine if other rare cutter enzymes generated a similar pattern of restriction sites to that found with NotI. BssHII, an enzyme expected to cleave especially in CpG islands, cleaved at positions 140, 190, 260, 350, 490, 600 in the 578 cell line (Fig. 3a). Furthermore NotI and BssHII both cleaved at similar positions at distances 140, 190, 260, 490 from the telomere, thus strongly suggesting CpG island-like structures.

2. Extension of the partial map from the telomere

a. Extension of the partial map with Not I to position 1900

The existence of a hypomethylated region containing many cleavable rare cutter restriction sites within the first 500 Kbp of the telomere has been demonstrated. There are no cleavable NotI sites between position 520 and 1250. At position 1250 and 1400 Kbp there are rare cutter sites like NotI (see Diagram. 1,A) and BssHII which give hints of an CpG island in this region.

Restriction digests of different male and female lymphoblastoid cell DNA's with NotI and separation of the generated partial restriction fragments under 'long gel' conditions allowed us also to distinguish between the hypomethylation cluster below 500 Kbp, the approx. 750 Kbp 'gap' of no sites and the next two NotI sites at positions 1250 and 1400 (see Fig. 4, e.g. track 1). Furthermore, the next NotI site follows at position 1900. The size of this band was deduced from the position of the two yeast markers at 1.4 Mb (S. cerevisia) and 3.0 Mb (S. pombe) and corresponded to the 9th identified NotI site from the telomere. In both male and female individuals the 1900 Kbp NotI fragment were of identical size and similar intensity. We concluded therefore that this site is still likely to be contained within the pseudoautosomal region.

b. Extension of the partial map with BssHII to 4 megabases

Despite a further enzyme reduction we could not observe partials extending beyond the 1900 Kbp NotI site. Therefore, we concentrated in our further analysis on the enzyme BssHII. Under optimal conditions (see Figure legends) partial fragments of 4000 kbp containing the telomere locus can be derived (see Fig 3c.). Again, the sizes of the fragments were deduced from the position of the S. cerevisiae marker and the two S.
Figure 3

Autoradiograms representing BssHII partial digestion of 578 ('X only') cell hybrid line DNA.

a. in the size range 0-1300 Kbp with fragments of 140, 190, 260, 350, 490, 600 and 1250 Kbp (partial bands 1-7)

b. in the size range of 0-3.5 Mbp with fragments 490, 600, 1250, 1400, 2400 Kbp (partial bands 5-9)c. in the size range 0-5 Mbp with fragments 1400, 2400, 3000, 4000 Kbp (partials 8-11).

Note that the smaller BssHII sites resolve only as a black solid block when the resolution gets shifted above 1500 Kbp (b,c) and also that in c only 1 band of around 1400 can get resolved, opposed to b where a distinction of two bands 1250 and 1400 are possible.

Enzyme amounts used were 20u, 5u, 2u, 1u and 0.5u (from left to right) for 4 hours (Fig. 3a), 0.5u and 0.25u (Fig 3b) (left 2 tracks shorter and right 2 tracks longer exposure) and 0.5u and 0.25u in Fig. 3c (left shorter and right longer exposure). Conditions of gel run see Material and Methods.

pombé markers of 3 and 5 Mbp length. Due to possible differences between the concentration of DNA of yeast relative to human lanes it is possible that the actual size of these fragments could differ from our estimates by 10%. Our experiences to date suggest that a difference of more than 10% of the predicted length is not very likely and that an overestimation is more likely than an underestimation of size. A comparison of the BssHII partials of the 853(Y) and 578(X) cell line, which provided the best DNA source for digestion with this enzyme showed that up to and including the 3 Mbp BssHII fragment (which represented the 10th BssHII site cleaved from the telomere) the BssHII fragments are the
Figure 4

Gel and autoradiogram of a series of different male and female lymphoblastoid cell lines, derived from different individuals, digested with NotI (enzyme concentration 1 and 0.5 units) and probed with 29A24. The chromosomes of *S. cerevisiae* (1.4 Mbp) and *pombe* (3 Mbp) have been used as molecular weight markers. As a control we have used *Hansenula wingei*, whose chromosomes provide molecular weight markers in the range below 3 Mpb, but the sizes of which are not known.

same on both the X and Y (see Fig. 3c). We conclude from this finding that the 3 Mbp fragment on the X and Y chromosome is still within the pseudoautosomal region. Diagram 1,C illustrates schematically all the BssHII sites detectable in the autorads over different range of resolutions (Fig 3a,b,c; Fig 5a, b). Whereas the smaller bands are well resolved in Fig. 3a, they form a black solid block on gels resolving larger fragments (b,c).

3. Estimate of the approximate size of the pseudoautosomal region

A comparison of the BssHII partial fragments in 578(X) and 853(Y) detected by 29A24 indicates that fragments up to and including 3Mbp are common between the X and Y chromosomes and therefore likely to be contained within the pseudoautosomal region (Fig. 3c). Beyond this position there is at least a strong hint of a map difference, possibly due to increased cleavage or additional well cleaved sites of slightly more than 3Mpb in the 853(Y) cell line. The first clearly visible difference on partial BssHII fragments of 853(Y) and 578(X) DNA is the appearance of a band at approximately 4 Mpb in 578(X), that cannot be detected in 853 (Y) (Fig. 3c). Since MIC2, a marker close to the proximal end of the
Figure 5

a. PFGE analysis of 853 DNA hybridised with 19b (MIC2). For gel run conditions see Material and Methods. Restriction enzymes were BssHII, SfiI, SalI, NotI/NruI double digests, NotI and NaeI. Yeast chromosomes provided the markers that were separated in the tracks at the ends of the gel. Sizes of 200-800 Kbp are shown. LM corresponds to the limiting mobility. Fragment sizes were 320 Kbp (BssHII); 20, 50, (200), (350) Kbp (SfiI), 350, (550), (680), (850) Kbp (SalI), 370 Kbp (NotI) and 80, (180), (260), (350) Kbp (NaeI) and the weakly cut fragment sizes are shown in brackets.

b. PFGE analysis of 578 DNA, hybridized with 19b (MIC2). The gel was run under conditions to resolve up to almost 1400 kbp. Restriction enzymes used were NaeI, NotI, PvuI, SacII, BssHII. As above yeast chromosomes provided the markers. Fragment sizes obtained were 350 Kbp (NaeI), 1300 Kbp (NotI), 200 and 280 Kbp (SacII) and 1200 and 1250 Kbp (BssHII) and PvuI fragments remained in the limiting mobility of the gel.

Pseudoautosomal region has been described (9) a map of the region around this probe should be compatible with the fragment sizes predicted from the partial BssHII digestions at the boundary from the X/Y shared to the X/Y specific region, visualized by the telomeric probe. Clone 19b (MIC2), which has been shown in blood and a cell line containing 4 Y
chromosomes (Oxen) to lie less than 200 Kbp away from the pseudoautosomal boundary (22) hybridises here to small Y derived 320 Kbp BssHII and 350 Kbp NotI fragments in 853(Y) and to 1200 and 1250 BssHII and 1300 Kbp NotI X derived fragments in 578(X). Thus these chromosomes can be clearly distinguished from each other (Fig. 5a, b). This result explains the almost unresolved double band around 3 Mbp in 853(Y) and the 1 Mbp difference between the 3 Mbp Y fragment in BssHII partials of 853(Y) and the 4 Mbp X fragment in BssHII partials of 578(X) (see also Diagram 1,C). Furthermore, the reason why we cannot get BssHII partials beyond approximately 3Mbp in 853(Y) in contrast to 578 (X) might reflect the high density of these sites in the proximal TDF CpG island (4 BssHII sites within 2.2 Kbp; 24) on the Y chromosome. If we assume that all the BssHII partials detected by MIC2 in 578 (X) (which are approximately 1200 Kb, 1800 Kbp in size) extend in a telomeric direction, the detected BssHII sites would also correspond to those responsible for the partial 2400 and 3000 Kbp fragments identified by 29A24 in both 578 (X) and 853 (Y) cell lines (data not shown), which should also be testable with other probes of that region.

DISCUSSION

To test the possibility of using telomere probes in the restriction mapping of ends of chromosomes by partial digestion and to establish a partial restriction map of the pseudoautosomal region of the human X and Y chromosomes, we took advantage of clone 29A24, located approximately 25 Kbp from the telomere (21).

After testing a number of 'rare cutter enzymes' of which some (SalI, MluI and NaeI) gave "natural partials" (even after extended digestion with high concentrations of enzyme many sites remain partially cleaved, an effect, which is likely to be due to partial methylation of the corresponding sites) we used reduced amounts of enzyme to generate partial digestion products. Among the enzymes tested, NotI and BssHII gave well controllable and reproducible partial digestion products and were therefore used in the further analysis.

Both enzymes show an unexpectedly high density of sites within the first 500 kbp, pointing to a both CpG rich and hypomethylated region. In male, female and 4X lymphoblastoid cell lines or male and female blood there are up to 7 NotI sites (see Diagram 1) and 5 BssHII sites in 578(X) within 500kbp from the telomere, also reminescent of the situation on
regions on the tip of chromosome 4, in which 12 Not sites are clustered within a distance of 400 kbp (Bucan, Zimmer et al., in preparation) or in the region around the α-globin cluster on the tip of chromosome 16 (25).

Such an extreme clustering of rare cutter sites, could either be due e.g. to a very high density of CpG islands containing genes in this region, or might reflect structural features of mammalian telomers, such as a high density of minisatellite-like sequences. Minisatellites from two different pseudo-autosomal loci have however been sequenced and found to display a very high A+T content (26).

In addition to raising questions about the functions of such clusters of CpG rich sequences, such an extreme clustering of sites has obvious implications in a number of techniques, relying on the use of rare cutter enzymes, as for example long range mapping, chromosome jumping (27, 28) and the use of rare cutter sites as aid in the localisation of genes (29, 30, 31).

Since cleavage by the rare cutter enzymes used in this analysis is blocked by CpG methylation, which is in turn correlated with gene activity (32) and expected to play a role in processes like X inactivation (33), mapping by partial digestion also offers information on differences in methylation patterns (or possible site polymorphisms) between different tissues and cell lines. In such analysis, blood and 3 different lymphoblastoid cell lines showed approximately the same degree of methylation, whereas in the 'Y only' cell line 853 and sperm about half of the sites were highly methylated (or not present) (Fig. 2a,b,c, Diagram 1). These results also confirm previous observations of a more relaxed methylation status in transformed cell lines in general (34, 35).

Since no differences between X and Y were observable on the gels separating NotI partial digest products extending up to 1.9 Mbp from the telomere, the analysis was extended to longer fragments, which was possible with the enzyme BssHII. A comparison of male and female lymphoblastoid cell lines or the 853(Y) and 578(X) cell lines shows another CpG island (cleaved by BssHII and NotI at position 1400) and again identical site positions on X and Y 1900 kbp from the telomere with NotI (Fig.4) and 2400 and 3000 kbp with BshII (Fig.3b,c, Diagram 1).

Although the number of genes associated with CpG islands (16) has yet to be determined, the low number of those in the pseudoautosomal region (not taking into consideration the hypomethylated area next to the telomere) could correspond to only a small number of genes in this region.
These identified potential CpG islands mentioned above could conceivably mark the positions of some candidate genes responsible for the Turner phenotype of XO females ("anti-Turner genes").

Using two different enzymes, and many different sources of DNA, no systematic differences between X and Y could be observed in the range till approximately 2000 kbp. In the range between 2000 and 4000 kbp the enzyme BssHII gave the same sized partial fragments at position 2400 and 3000 kbp on the two cell lines 853(Y) and 578(X). We concluded therefore that the 3000 kbp BssHII fragment is likely to be still localised within the pseudoautosomal region (Fig3). Differences between X and Y are however visible beyond a point of approximately 3 Mbp from the end of the chromosome, shown on the Y only and X only hybrid cell lines (since the absence of a band in Y would not be easily detectable in the comparison between XX and XY DNA). Although the alternative explanation of a site or methylation polymorphism within the pseudoautosomal region cannot completely ruled out, this difference would position the expected end of the pseudoautosomal region at approximately 3000 kbp. This is an assignment also compatible with both the sizes of fragments found to hybridise with MIC2 in complete digests, and the observation of a high density of BssHII sites in the CpG island next to the TDF gene (24), located close to the end of the pseudoautosomal region and offering an possible explanation for the absence of partial digestion products extending beyond this point in the Y only hybrid.

The physical length of the pseudoautosomal region of approximately 3 Mbp correlates well with the genetical average of 5% recombination frequency in female meiosis. The greatly increased recombination rates observed in males is associated with a single obligatory meiotic crossing over within a very limited genomic segment. That the relationship between physical distance and recombination frequencies calculated as an average for the genome as a whole holds also true for the pseudoautosomal region in females, argues against a strongly enhanced rate of exchange near telomers in general.

An extension of the range of long range maps and a further improvement of partial digest conditions obviously make the use of probes derived from mammalian telomeres very attractive. Cloned telomeric repeat sequences from Tetrahymena thermophila which are highly conserved within species and cross-hybridise to the telomeres of most, if not all, human chromosomes (36) will make it potentially possible
to establish long range maps of all the ends of mammalian chromosomes, offering easy access to physical maps, which cover a significant fraction of the mammalian genome.

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