Human Molecular Genetics, 1997, Vol. 6, No. 11 **1985–1989**

Abnormal XY interchange between a novel isolated protein kinase gene, *PRKY*, and its homologue, *PRKX*, accounts for one third of all (Y+)XX males and (Y–)XY females

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Received June 27, 1997; Revised and Accepted July 31, 1997

DDBJ/EMBL/GenBankAccession Nos Y13927–Y13934

XX males and XY females have a sex reversal disorder which can be caused by an abnormal interchange between the X and the Y chromosomes. We have isolated and characterized a novel gene on the Y chromosome, *PRKY*. This gene is highly homologous to a previously isolated gene from Xp22.3, *PRKX*, and represents a member of the cAMP-dependent serine threonine protein kinase gene family. Abnormal interchange can occur anywhere on Xp/Yp proximal to *SRY*. We can show that abnormal interchange happens particularly frequently between *PRKX* and *PRKY*. In a collection of 26 XX males and four XY females, between 27 and 35% of the interchanges take place between *PRK* homologues but at different sites within the gene. *PRKY* and *PRKX* are located far from the pseudoautosomal region where XY exchange normally takes place. The unprecedented high sequence identity and identical orientation of *PRKY* to its homologous partner on the X chromosome, *PRKX*, explains the high frequency of abnormal pairing and subsequent ectopic recombination, leading to XX males and XY females *and* to the highest rate of recombination outside the pseudoautosomal region.

INTRODUCTION

The XX male syndrome (1) and Swyer's syndrome (XY gonadal dysgenesis) (2) are two genetic disorders interfering with normal gonadal development in humans. XX males resemble individuals with Klinefelter's syndrome (47,XXY) in their general masculine appearance. They are infertile and have small testes. Conversely, individuals with Swyer's syndrome are phenotypic females with streak gonads, amenorrhea and a male karyotype (46,XY). The XX male syndrome has an incidence of 1:20 000 in the general population, while Swyer's syndrome occurs more rarely (3). In ~80% of XX males, the male phenotype is caused by the presence of Y-specific DNA, including *SRY* (4–11), whereas in XY females, at a lower percentage, the phenotype is due to the absence of Y-specific DNA (12–16). The abnormal exchange between the X and Y chromosomes has been characterized in both XX males and XY females as a terminal exchange (10,11,16).

Two hot spots of recombination (HSA and HSB) leading to a high frequency of XX males and XY females have been described previously and positioned in the interval 3F and 3G on the human Y chromosome (17–19). Here we report results on the isolation of a 250 kb PAC and cosmid contig and on the characterization of the Y homologue of the protein kinase gene *PRKX* within this contig, which will be referred to as *PRKY* (20). We analysed the Y-chromosomal breakpoints of 26 XX males and four XY females, who were selected because their breakpoints previously were roughly mapped and shown to reside on interval 3 (17–20). We can show that the breakpoints of all tested XX males and XY females either reside within the genomic locus of *PRKY* or in the direct vicinity of *PRKY*. This implies that the *PRKY*-containing subregion exhibits the highest recombination rate on the Y-specific portion of the Y chromosome.

RESULTS

PRKX cDNA fragments were used to isolate homologous sequences on the Y chromosome. For this purpose, a Y-sorted Lawrence Livermore cosmid library LLOYNC03 'M' and a human male PAC library (21) were hybridized with *PRKX* cDNA subfragments (20; see also Materials and Methods), resulting in five overlapping cosmids and two flanking PACs. Sequencing with *PRKX*-derived primers and PCR analysis with sequence-tagged sites (STSs) *DYS263* (sY68), *DXYS163Y* (HSB), *DYS264* (sY69) and *DXYS164Y* (HSA) and the

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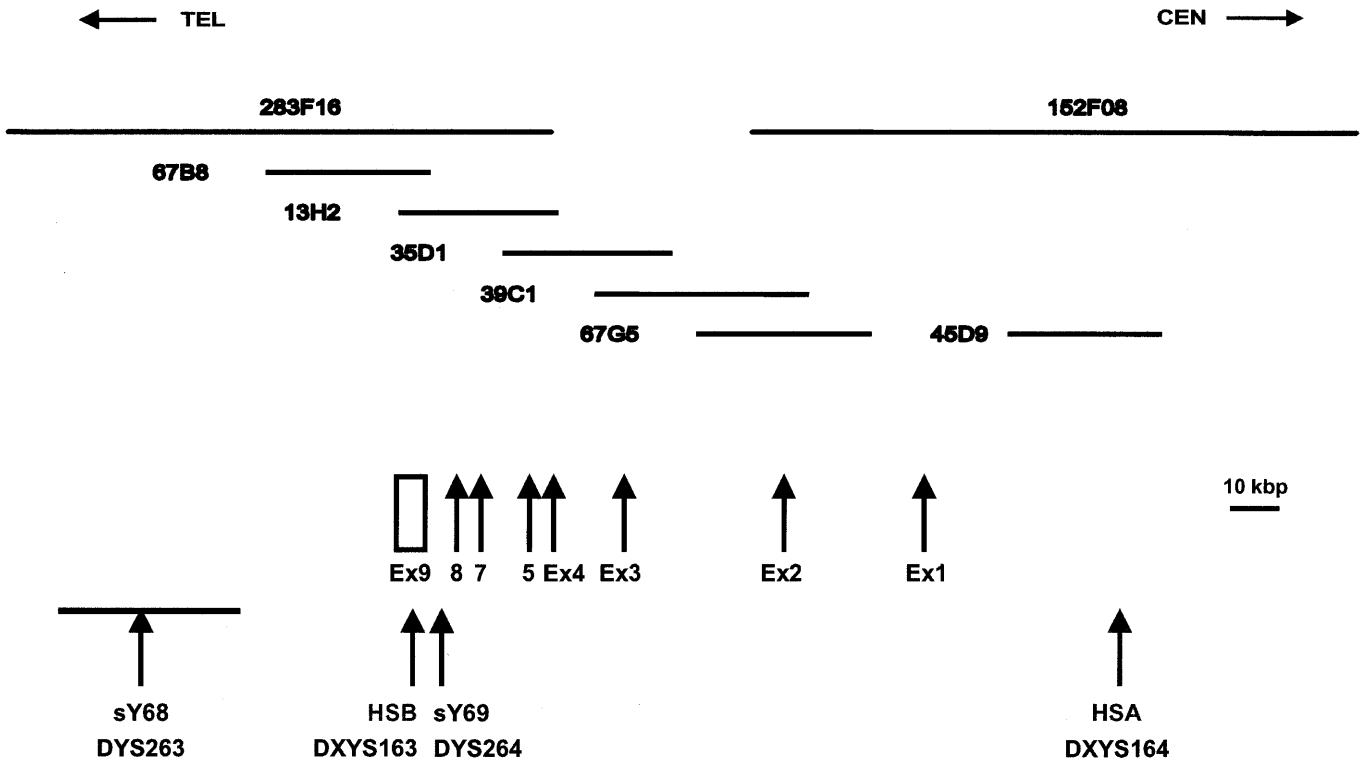

Figure 1. Schematic diagram of the PAC/cosmid contig from interval 3G/3F on Yp and the genomic organization of *PRKY*. Exons were numbered in accordance with *PRKX*. The position of markers sY68 (*DYS263*), sY69 (*DYS264*) (17), HSB (*DXYS163*) and HSA (*DXYS164*) (18) are indicated on the map; HSA/HSB: hot spot of recombination A/B. PACs 283F16 and 152F08 were mapped by FISH: both showed strong Yp11.2 signals and weaker signals on the Xp22.3 region (unpublished results). This phenomenon was also seen with the two tested cosmids 67B8 and 67G5 of the region.

hybridization of the amplification products to Southern blots of digested cosmid and PAC DNA allowed the construction of the map depicted in Figure 1. Partial Y-specific cDNAs were derived from fetal brain and bone marrow cDNA libraries at high stringency screening (see Materials and Methods). Y-specific exons were also identified based on their similarity to *PRKX* (formerly *PKX1*, 20) using X-specific primers and by sequencing Y-specific cosmids and PACs from the region. The known order of sY markers on the YAC map of Foote *et al*. (22) revealed that the 5′→3′ orientation of *PRKY* is towards the telomere. *PRKY* is composed of eight exons with length ranging from 78 to 6047 bp (Fig. 1). The last exon represents a block of ~6 kb and starts 22 bp downstream of the stop codon in *PRKX* cDNA, and thus represents the 3′-untranslated region (UTR). Due to a 1.4 kb insertion and several small gaps, this region is 1.3 kb longer in *PRKY* than in *PRKX*. The polyadenylation signal sequence, however, is located at the corresponding position (EMBL accession Nos X85545 and Y13934). Sequence comparison with *PRKX* cDNA revealed that exon 1 of *PRKY* is composed of 504 bp, with 338 bp representing the 5′ UTR. An exon corresponding to nucleotides 1182–1239 in *PRKX* cDNA is missing in *PRKY*. Despite the conservation of start and stop codons at the corresponding positions in both *PRKX* and *PRKY* and the conservation of the open reading frame (ORF) in all existing exons, the predicted protein of *PRKY* is 81 amino acids shorter than that of *PRKX*. This is due to a frameshift caused by the loss of the smallest 58 bp exon. The deletion leading to the loss of exon 6 seems to be a very recent event during evolution, since no additional frameshift or stop mutations have accumulated in any other exons. In contrast, a pseudogene, *PRKXP1*, which maps to 15q26, was shown to be truncated not only by a stop codon but also by the insertion of two different repeats (23).

Breakpoint analysis of XX males and XY females

The two largest collections of XX males and XY females with known breakpoints in interval 3F and 3G on Yp were investigated in order to define the breakpoint regions with respect to *PRKY* (17,19,24). Using *PRKY* exon-specific primers and primers flanking the gene on either side we show in Figure 2 that 13/30 deletion breakpoints occur intragenically (within the 110 kb genomic locus of *PRKY*), two of the deletion breakpoints reside close to exon 9 (WHT1209 and WHT1870) and 15 deletion breakpoints are upstream to exon 1, 12 of which reside in a distance of 30 kb in the previously described hot spot HSA (18). The distribution of translocation breakpoints reveals that five different intervals appear to be particularly prone to this type of rearrangement: translocation breakpoints within *PRKY* occur between exons 1 and 2 (patient 693), between exons 3 and 5 (LGL105, GA001, WHT304, LGL850/WHT438 and WHT1327), between exons 5 and 8 (CHM347 and 651), between 9A and 9B in exon 9 in the 3′ UTR (WHT1856, GA/WHT1123, TA, NAN194 and PAR089), between exon 1 and HSA (WHT1010) and in HSA ~30 kb upstream of exon 1 (Figs 1 and 2). The breakpoint regions of two patients WHT950 and WHT2127 were narrowed down to a 650 bp fragment using an

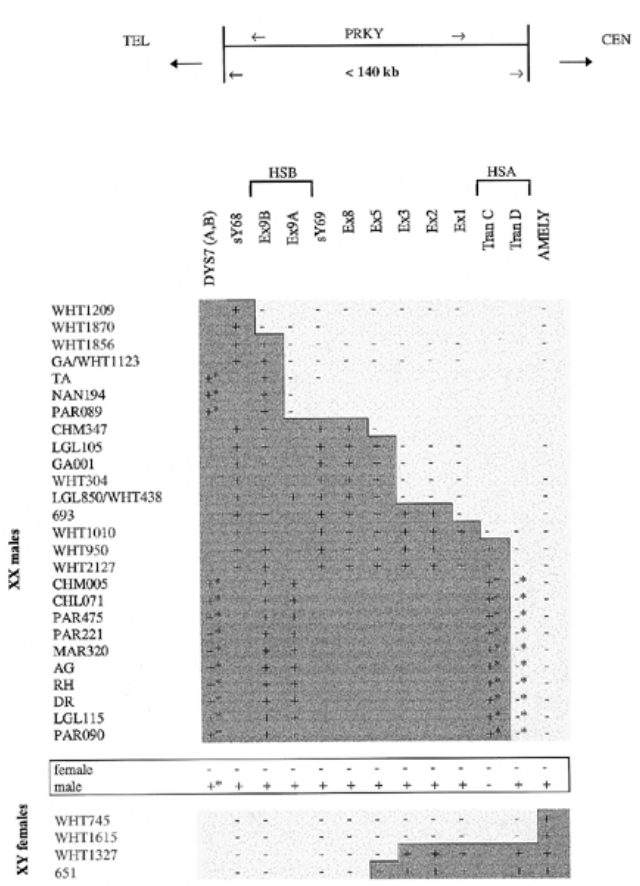

Figure 2. Deletion map of the *PRKY* gene region on the short arm of the Y chromosome. Listed on the left are those 26 XX male and four XY female individuals analysed, who carry deletion breakpoints between sY68 and *AMELY*. One normal male and one normal female were used as controls. WHT1209, WHT950, WHT1010, WHT2127, WHT745, WHT1615 and WHT1327 are from the series of Vollrath *et al*. (17). XX male WB from the original series (17) was omitted, as his breakpoint could be redefined distal to sY68 (data not shown) and was substituted by WHT1870, WHT1856 and WHT304. TA, NAN194, PAR089, CHM347, GA001, CHM005, CHL071, PAR475, PAR221, MAR320, AG, RH, DR, PAR090 and LGL850 are from the patient series of Wang *et al*. (19); GA/WHT1123, LGL105, LGL115 and LGL850/WHT438 are contained in both collections; 651 and 693 have been described in Klink *et al*. (20). Across the top, one hybridization probe and 12 STSs are listed, eight of which represent ESTs from *PRKY* (exons were numbered in accordance with *PRKX*). Eleven STSs (sY68–TranD) lie in a genomic interval of <140 kb ~7 Mbp from the Yp–telomere (Yp–tel distance is based on available YAC contigs) (33). The short arm telomere (TEL) is to the left and the centromere (CEN) to the right. HSA and HSB are recombination hot spots (18). The presence or absence of loci was detected by PCR. The experimentally demonstrated presence of a locus is indicated by +, its absence by a minus and the inferred absence/presence of a locus is not marked at all. Patients with an asterisk: *DYS7*, *TranD* and *TranC* loci have been tested previously by hybridization (19). Note that TranCX1for is an X-specific primer used for the amplification of the X–Y junction fragment (see Materials and Methods).

X-specific forward primer and a Y-specific reverse primer (see Materials and Methods; TranC amplification). In addition, the amplification and subsequent sequencing of the breakpoint region in these two patients also revealed that the recombination is truly homologous (Fig. 3).

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       1 GGATCCGATTCCTTGCCTTCTTTAGCTTCCAGCGTTTCCAAGCTCATGGC  50
WHT950   -------X----X------X-----X-------X****************
WHT2127  -------X----X------X-----X-------X****************

      51 TCTCCCTCACTCGCATCTCAGCTTCCATTTTCATGTCTCCTTCTCCAACT 100
WHT950   **************************************************
WHT2127  **************************************************

     101 TGGACCCTCCTGCCTCCCTCTTAGAAGGAGCCTGTGACGGCATTTAGGGC 150
WHT95    **************************************************
WHT2127  **************************************************

     151 CAACCTGGGTCATCCAGGGGCATCTCCCATCTCAAGATCCTTAACCTCAT 200
WHT950   **************************************************
WHT2127  **************************************************

     201 CTCATCTGCAAAGTCCTCCTGACCGTGCAAGATCAGCTGTTCCCAGGTTC 250
WHT950   **************************************************
WHT2127  ***************p**********************************

     251 CAGGGATTCAGAT GTGGACATCTTTGGGGAATCATTATTCTACCTATCA 300
WHT950   ******************************yy-----------------
WHT2127  ******************************yy-----------------

     301 CAGTA  305
WHT950   -----
WHT2127  -----
```

Figure 3. Comparison of DNA sequences of patients and hot spot A sequence (18). The sequence starts at the *Bam*HI site and is numbered according to HSTRANXYC (accession No. X70412; breakpoint region sequenced from the X chromosome). DNA sequences of the patients are indicated by symbols: – nucleotide identical in X,Y and patient DNA; x nucleotide identical in X and patient DNA, y nucleotide identical in Y and patient DNA, p polymorphic nucleotide (in patient DNA but neither in X nor in Y DNA); * nucleotide of breakpoint region (identical in X and Y DNA).

In summary, the previously described recombination hot spots A and B have a distance of maximally 140 kb on the Y chromosome and enclose the *PRKY* gene. The extensive XY homology of *PRKX* and *PRKY* genes leads to abnormal XY interchange in at least 33 (>17/52 based on ref. 19) to 35% (8/23 based on ref. 17) of all (Y+)XX males and in 27% (3/11 based on ref. 17) of all (Y–)XY females.

DISCUSSION

Recombination between X and Y chromosomes is normally restricted to the pseudoautosomal regions located at both ends of the sex chromosomes, where pairing of X and Y chromosomes also initiates. An obligate crossover in the pseudoautosomal region (PAR1) during male meiosis seems to be necessary for male fertility (25). The sequence identity between X and Y chromosomes found within the PAR1 is interrupted abruptly at the pseudoautosomal boundary where X- and Y-specific regions begin. On the Y chromosome, this region harbours the testis-determining gene, *SRY* (26). Recombination occurring outside the pseudoautosomal region in the Xp–Yp homologous regions results in a translocation of the *SRY* gene from the Y to the X chromosome, and consequently to the development of (Y+)XX males or (Y–)XY females. Illegitimate recombination involving Xp–Yq homologous regions has also been described as the cause of Xp22.3–Yq11 translocations, but these seem to be extremely rare (27,28). We show that abnormal interchange happens particularly frequently between two small X–Y homologous regions harbouring the *PRKX*/*PRKY* genomic loci.

PRKY and *PRKX* have a high overall sequence similarity of 94% and encode proteins with an intact ATP-binding domain and a catalytic domain with high homology to protein kinases. *PRKY* and *PRKX* are expressed at different levels (data not shown), and we do not know if both proteins are functional. Twelve differences in amino acids between PRKY and PRKX fall in the first exon, and the putative promotor region shows only 89% sequence similarity, suggesting that potential differences in transcription activity and functional relevance probably reside in the respective 5′ portions of the genes. The shortening of the putative PRKY protein results in the loss of a highly conserved arginine (named Arg280), which was shown to interact with a glutamic acid (Glu208) and seems to play an important role in the formation of the three-dimensional structure of all protein kinases investigated so far (29). On the other hand, several members of the protein kinase family are known to have short C-termini similar to the *PRKY* product (30). Further biochemical investigations are needed to demonstrate whether both transcripts are translated and have similar or different functions.

What is the reason for this frequent occurrence of abnormal XY interchange intragenically or directly adjacent to *PRKY*, ~7 Mbp proximal to PAR1 on Yp (31)? To our knowledge, the two other known Xp–Yp gene pairs, *ZFX/ZFY* and *AMELX/AMELY*, with sequence similarities of 92 and 91%, respectively, are not prone to ectopic recombination. Whereas in *AMELX/AMELY* high similarities between exons and the flanking intron regions have also been noticed, gene loci are short (10 kb) and the orientation of both genes is unknown (32). In comparison, *ZFX*/*ZFY* are in the same orientation on X and Y, cover larger genomic loci (70 and 50 kb), yet sequence homology seems to be restricted to the exons and the CpG island region at the 5′ ends (33). Thus, it appears that the biology of the abnormal interchange is implicated by a combination of factors such as the high sequence similarity of *PRKY* to *PRKX* (94% homology) extending into the introns, the unusually long untranscribed last exon (6 kb/4.7 kb) with 93% identity, the large genomic locus of *PRKY* (~110 kb) and the identical orientation of both genes on the sex chromosomes. The high incidence of ectopic recombination in the *PRKX/PRKY* gene regions in *both* XX males *and* XY females also demonstrates that (Y–)XY females represent true mirror images of (Y+)XX males, as originally hypothesized by Ferguson-Smith in 1966 (4).

MATERIALS AND METHODS

Patients

XX males and XY females patients are from two large series described by Vollrath *et al.* (17) and Wang *et al.* (19). Patients 693 and 651 have been described by Klink *et al.* (20).

cDNA isolation

Two fetal brain cDNA libraries (Stratagene 937227; 1.2×10^6 clones and Clontech HL3003b; 0.9×10^6 clones; mixed male and female tissue), a placenta (Stratagene 936203; 0.75×10^6 clones; male tissue), a bone marrow (Clontech HL1058b; 0.75×10^6 clones; male tissue) and a testis cDNA library (Clontech HL3024b; 1.2×10^6 clones; male tissue) of human origin were screened with *PRKX* cDNA fragments according to standard techniques. Final stringencies of washes were 1% SDS, 20 mM Na_2HPO_3 at 60°C. Pre-selection of Y-specific cDNA clones was done by PCR amplification of plaques with primer 166cyfor: CCA AGC ATT TCT TCG CCC and 166cyrev: CGT GAG CTT **G**AT GTG **A**CC (annealing at 55°C) followed by a *Pst*I digestion. The 356 bp amplification products from Y chromosomal transcripts cannot be cleaved with *Pst*I to 248 and 108 bp fragments, in contrast to the X-derived transcript.

Three Y-specific partial cDNA clones were derived from the Clontech fetal brain and the bone marrow cDNA libraries extending from 577 to 834 bp (exon 2 + partial exon 3), 639 to 894 bp (partial exon 2 + partial exon 3) and 577 to 966 (exon 2 + exon 3). Localization refers to the *PRKX* cDNA sequence (accession No. X85545).

Cosmid and PAC screening

PRKX cDNA fragments (0.77 and 0.69 kb *Sac*I subfragments of FB166) and a genomic 4.5 kb *Bam*HI–*Sac*II fragment enclosing the 3′ UTR (20) were hybridized to gridded filters of the Y-sorted Lawrence Livermore cosmid library LLOYNC03 'M' (constructed by J. Garnes and P. de Jong) and to a human male PAC library (21). Final wash conditions were 1% SDS, 20 mM Na_2HPO_3. The hybridization and wash temperature was reduced from 65 to 58°C for exon 1. Localization of PACs and several cosmids was confirmed by fluorescence *in situ* hybridization which was carried out as previously described (20).

Sequencing

DNA double-strand sequencing of PCR products cloned in pMOS-vector (Amersham) was performed with an ALF express automated sequencer (Pharmacia).

Primers and PCR conditions for breakpoint analysis

PCRs were performed in 50 µl volumes containing 20–100 ng of genomic template DNA, 50 pmol of each primer, 200 µM dNTP, 5% dimethylsulphoxide (DMSO), buffer and $MgCl_2$ as supplied with *Taq* polymerase (Eurogentec). Cycling was carried out in a Thermocycler 60 (Biomed): 1 min at 94°C, 30 s at the annealing temperature (see below), 30 s at 72°C.

Primer sequences, annealing temperatures and fragment length were as follows: sY68, sY69 and AMELY see ref. 17, annealing at 62, 64 and 62°C, respectively; exon 9B: FB1Yfor, GAC CTT TTC TTC ACG TG**AC**; FB1Yrev, AAA ACA GAC AAC **A**TA AAA **T**TA **CA**, 58°C annealing, 578 bp; exon9A: 166A2Yfor, CAA G**AC** TTT CTT C**T**C C**AC** C; 166A2Yrev, **C**AT TT**C** CCT **TG**A CAT TTT GC, 58°C annealing, 364 bp; exon 8: pky8for, TCG TGC CCA **C**GA TGA CTG G**CA**; pkyrev, TCT TCC AGA TGT GAGF CTC **..**GTC C, 67°C annealing, 139 bp; exon 5: pkyI4 for, ACA ACT TCA **A**TG TG**T** GGG GAA GA**A**; pky5rev, AAT CCA AAT GTC TGG GGA AAT **A**TA **G**, 60°C annealing, 228 bp; exon 3: 166cy3for, CTC ATG GAG TA**T** GTG CCG GG**T**; 166cy2rev, GTC CGT GAG CTT **G**AT GTG **A**CC, 65°C annealing, 197 bp; exon 2: 166cy2for, **GG**A AGC AGG A**G**C AGC ACG T**G**; pkyI2Brev, GAG TGC GTC GGG **A**GA GGC **C**, 65°C annealing, 108 bp; exon 1: pkyE1for, ACT CCC G**AG** AGG TG**A** CGG; pky1rev, CA**T** GGT G**A**C CAG CG**C** GTC **GC**, 62°C annealing, 121 bp; TranD: TranDfor, CC**.**T GCC TT**T** TTT AG**T** TTC CAG C**A**; TranDrev, TAC TGT GAT AGG TAG AAT AAT G**GC**, 63°C annealing, 292 bp; TranC: TranCX1for, GGG **CAC A**GT GGT TCA CAC **TG**; TranDrev, see above, annealing, 63 °C, 649 bp. Bases in bold indicate Y-specific bases (versus XY homologous bases); dots mark

missing bases in the Y copy. Note that all primers are Y-specific except for TranCX1for, which is an X-specific primer used for the amplification of the X–Y junction fragment (here bases in bold indicate X-specific bases).

ACKNOWLEDGEMENTS

We thank Pieter de Jong for the PAC and cosmid libraries (constructed at the HGC, LLNL, Livermore, CA 94550 under the auspices of the National Laboratory Gene Library Project sponsored by U.S. DOE). We also thank Jeff Craig, Anne Jordan, Albrecht Klink, Birgit Schechinger, Gerd Scherer, Laura Brown and Raaji Alapaggan for help and critical comments and C.R. Bartram for his support. The research was supported by the Deutsche Forschungsgemeinschaft RA 380/9-1 and the Medizinische Fakultät, Universität Heidelberg.

REFERENCES

1. de la Chapelle, A., Hortling, H., Niemi, M. and Wennström, J. (1964) XX sex chromosomes in a human male. First case. *Acta Med. Scand*., **412**, 25–38.
2. Swyer, G.M. (1955) Male pseudohermaphroditism: a hitherto undescribed form. *Br. Med. J*., **2**, 709–712.
3. de la Chapelle, A. (1981) Etiology of maleness in XX men. *Hum. Genet.*, **58**, 105–116.
4. Ferguson-Smith, M.A. (1966) X–Y chromosomal interchange in the etiology of true hermaphroditism and of XX Klinefelter's syndrome. *Lancet*, **ii**, 475–476.
5. Guellaen, G., Casanova, M., Bishop, C., Geldwerth, D., Andre, G., Fellous, M. and Weissenbach, J. (1984) Human XX males with Y single copy DNA fragments. *Nature*, **307**, 172–173.
6. Page, D.C., de la Chapelle, A. and Weissenbach, J. (1985) Chromosome Y-specific DNA in related human XX males. *Nature*, **315**, 224–226.
7. Affara, N.A. Ferguson-Smith, M.A., Tolmie, J., Kwok, K., Mitchell, M., Jamieson, D., Cooke, A. and Florentin, L. (1986) Variable transfer of Y-specific sequences in XX males. *Nucleic Acids Res*., **14**, 5375–5387.
8. Andersson, M., Page, D.C. and de la Chapelle, A. (1986) Chromosome Y-specific DNA transferred to the short arm of X chromosome in human XX males. *Science*, **233**, 786–788.
9. Müller, U., Donlon, T., Schmid, M., Fitch, N., Richer, C.L., Lalande, M. and Latt, S.A. (1986) 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.
10. Page, D.C., Brown, L.G. and de la Chapelle, A. (1987) Exchange of terminal portions of X- and Y-chromosomal short arms in human XX males. *Nature*, **328**, 437–440.
11. Petit, C., de la Chapelle, A., Levilliers, J., Castillo, S., Noel, B. and Weissenbach, J. (1987) An abnormal terminal X–Y interchange accounts for most but not all cases of human XX maleness. *Cell*, **49**, 595–602.
12. Disteche, C.M., Casanova, M., Saal, H., Friedman, C., Sybert, V., Graham, J., Thuline, H., Page, D.C. and Fellous, M. (1986) Small deletions of the short arm of the Y chromosome in 46,XY females. *Proc. Natl Acad. Sci. USA*, **83**, 7841–7844.
13. Müller, U. (1987) Mapping of testis-determining locus on Yp by the molecular genetic analysis of XX males and XY females. *Development*, **101**, 51–58.
14. Affara, N.A., Ferguson-Smith, M.A., Magenis, R.E., Tolmie, J.L., Boyd, E., Cooke, A., Jamieson, D., Kwok, K., Mitchell, M. and Snadden, L. (1987) Mapping the testis determinants by an analysis of Y-specific sequences in males with apparent XX and XO karyotypes and females with XY karyotypes. *Nucleic Acids Res.*, **15**, 7325–7342.
15. Ferguson-Smith, M.A., Affara, N.A. and Magenis, R.E. (1987) Ordering of Y-specific sequences by deletion mapping and analysis of X-Y interchange males and females. *Development*, **101**, 41–50.
16. Levilliers, J., Quack, B., Weissenbach, J. and Petit, C. (1989) Exchange of terminal portions of X- and Y-chromosomal short arms in human XY females. *Proc. Natl Acad. Sci. USA*, **86**, 2296–2300.
17. Vollrath, D., Foote, S., Hilton, A., Brown, L.G., Beer-Romero, P., Bogan, J.S. and Page, D. (1992) The human Y chromosome: 43-interval map based on naturally occurring deletions. *Science*, **258**, 52–59.
18. Weil, D., Wang, I., Dietrich, A., Poustka, A., Weissenbach, J. and Petit, C. (1994) Highly homologous loci on the X and Y chromosomes are hot-spots for ectopic recombination resulting in XX maleness. *Nature Genet.*, **7**, 414–419.
19. Wang, I., Weil, D., Levilliers, J., Affara, N., de la Chapelle, A. and Petit, C. (1995) Prevalence and molecular analysis of two hot spots of ectopic recombination leading to XX maleness. *Genomics*, **28**, 52–58.
20. Klink, A., Schiebel, K., Winkelmann, M., Rao, E., Horsthemke, B., Lüdecke, H.-J., Claussen, U., Scherer, G. and Rappold, G. (1995) The human protein kinase gene PKX1 on Xp22.3 displays Xp/Yp homology and is a site of chromosomal instability. *Hum. Mol. Genet.*, **4**, 869–878.
21. Ioannou, P.A., Amemiya, C.T., Garnes, J., Kroisel, P.M., Shizuya, H., Chen, C., Batzer, M.A. and de Jong, P.J. (1994) A new bacteriophage P1-derived vector for the propagation of large human DNA fragments. *Nature Genet.*, **6**, 84–89.
22. Foote, S., Vollrath, D., Hilton, A. and Page, D.C. (1992) The human Y chromosome: overlapping DNA clones spanning the euchromatic region. *Science*, **258**, 60–66.
23. Schiebel, K., Mertz, A., Winkelmann, M., Gläser, B., Schempp, W. and Rappold, G. (1997) FISH localization of the human Y-homolog of protein kinase *PRKX (PRKY*) to Yp11.2 and two pseudogenes to 15q26 and Xq12–q13. *Cytogenet. Cell Genet*., **76**, 49–52.
24. Vergnaud, G., Page, D.C., Simmler, M.C., Brown, L., Rouyer, F., Noel, B., Botstein, D., de la Chapelle, A. and Weissenbach, J. (1986) A deletion map of the human Y chromosome based on DNA hybridization. *Am. J. Hum. Genet*., **38**, 109–124.
25. Rouyer, F., Simmler, M.C., Vergnaud, G., Johnsson, C., Levilliers, J., Petit, C. and Weissenbach, J. (1986) A gradient of sex linkage in the pseudoautosomal region of the human sex chromosomes. *Cold Spring Harbor Symp. Quant. Biol.*, **51**, 221–228.
26. Sinclair, A.H., Berta, P., Palmer, M.S., Hawkins, J.R., Griffiths, B.L., Smith, M.J., Foster, J.W., Frischauf, A.M., Lovell-Badge, R. and Goodfellow, P.N. (1990) A gene from the human sex-determining region encodes a protein with homology to a conserved DNA-binding motif. *Nature*, **346**, 240–244.
27. Yen, P., Marsh, B., Allen, E., Tsai, S.P., Ellison, J., Connolly, L., Neiswanger, K. and Shapiro, L.J. (1988) The human X-linked steroid sulfatase gene and a Y-encoded pseudogene: evidence for an inversion of the Y-chromosome during primate evolution. *Cell*, **55**, 1123–1135.
28. Guioli, S., Incerti, B., Zanaria, E., Bardoni, B., Franco, B., Taylor, K., Ballabio, A. and Camarino, G. (1992) Kallmann syndrome due to a translocation in an X/Y fusion gene. *Nature Genet*., **1**, 337–340.
29. Knighton, D.R., Bell, S.M., Zheng, J., Ten Eyck, L.F., Xuong, N.-H. and Taylor, S.S. (1993) 2.0 A refined crystal structure of the catalytic subunit of cAMP-dependant protein kinase complexed with a peptid inhibitor and detergent. *Acta Crystallogr*., **D49**, 357–361.
30. Hanks, S.K., Quinn, A.M. and Hunter, T. (1988) The protein kinase family: conserved features and deduced phylogeny of the catalytic domains. *Science*, **241**, 42–52.
31. Affara, N., Bishop, C., Brown, W., Cooke, H., Davey, P., Ellis, N., Graves, J.A.M., Jones, M., Mitchell, M., Rappold, G., Tyler-Smith, C., Yen, P. and Lau, Y.-F.C. (1996) Report on the second international workshop on Y chromosome mapping 1995. *Cytogenet. Cell Genet.*, **73**, 33–76.
32. Salido, E.C., Yen, P.H., Koprivnikar, K., Yu, L-U. and Shapiro, L.J. (1992) The human enamel protein gene amelogenin is expressed from both the X and Y chromosomes. *Am. J. Hum. Genet.*, **50**, 303–306.
33. Luoh, S.-W., Jegalian, K., Lee, A., Chen, E.Y., Ridley, A. and Page, D.C. (1995) CpG islands in human ZFX and ZFY and mouse ZFX genes: sequence similarities and methylation differences. *Genomics*, **29**, 353–363.