A Sex Chromosome Rearrangement in a Human XX Male Caused by Alu–Alu Recombination

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Summary

Human XX maleness is often due to the presence of Y-specific DNA, resulting from abnormal interchange of terminal parts of the short arms of the X and Y chromosomes. In an XX male, a rearrangement is observed at locus DXYS5, the most proximal Yp locus detected in this patient. Cloning and analysis of the rearranged DNA fragment revealed pseudoautosomal sequences located beyond the breakpoint. We propose that this XX male arose by abnormal crossing over between DXYS5 on the Y chromosome and a pseudoautosomal locus on the X chromosome during paternal meiosis. Sequence analysis of the junction shows that homologous recombination occurred between two Alu sequences from these otherwise nonhomologous regions. The site of recombination is localized to the putative transcription promoter region of the Alu sequences.

Introduction

Terminal portions of the short arms of the human X and Y chromosomes are strictly homologous and undergo crossing over (Cooke et al., 1985; Simmler et al., 1985; Rouyer et al., 1986a, 1986b; Goodfellow et al., 1986; Page et al., 1987b). Because of their autosome-like inheritance, loci from this homologous region are termed "pseudoautosomal" (Burgoyne, 1982). Pairing of the short arms of the X and Y chromosomes in male meiosis extends far beyond this region of pseudoautosomal homology (Chandley et al., 1984; Burgoyne, 1986). Genetic linkage analysis has shown that a single and obligatory X–Y crossing over takes place at variable locations during male meiosis and hence defines a gradient of sex linkage in the pseudoautosomal region (Rouyer et al., 1986a, 1986b). The obligatory character of the male pseudoautosomal crossover explains the 10- to 20-fold male versus female increase in the recombination frequency observed in this region. The high recombinational activity observed in the pseudoautosomal region could be related to the existence of anomalous recombination events involving loci from the nonhomologous part of the pairing region (Weissenbach et al., 1987).

Ferguson-Smith (1966) proposed that an abnormal interchange between the human X and Y chromosomes at paternal meiosis could be the cause of XX maleness. Human XX males are sterile men with a 46,XX karyotype; most cases occur sporadically, at a frequency of about 1 per 20,000 males (reviewed in de la Chapelle, 1981). The abnormal X–Y interchange would result in the transfer of a part of the Y chromosome including the gene(s) controlling testis differentiation. This testis determining factor (TDF) exerts a dominant effect on the differentiation of the primordial gonad. The use of Y-specific DNA probes demonstrated, first, that variable amounts of Y-derived material are present in XX males (Y(+)XX maleness) (Guellaën et al., 1984; Page et al., 1985); and second, that TDF is located in the distal part of the Y chromosome short arm (Vergnaud et al., 1986; Affara et al., 1986; Müller et al., 1986; Page, 1986). Furthermore, in situ hybridization experiments demonstrated that Y-specific DNA was carried by one of the X chromosomes in XX males (Andersson et al., 1986; Buckle et al., 1987).

The terminal-interchange model has been verified by analysis of the inheritance of pseudoautosomal loci in human XX males (Petit et al., 1987; Page et al., 1987a). These studies show that all (Y(+)XX males tested inherited the entire pseudoautosomal region from the paternal Y chromosome and that most lost the pseudoautosomal region from the paternal X chromosome. However, one XX male inherited not only the entire pseudoautosomal region of the paternal Y but also a proximal portion of the pseudoautosomal region of the paternal X. In this XX male, the recombination point on the X is likely to lie within the pseudoautosomal region (Page et al., 1987a). Taken together, these studies show that in Y(+)XX males the positions of both the Yp and Xp breakpoints are variable. All eight Y(+)XX males examined to date apparently result from single erroneous recombination events between the X and Y chromosomes. As mentioned above, such an event may thus take place instead of the normal X–Y crossover (Polani, 1982), but the molecular basis of this abnormal interchange is not yet defined. An analysis of X–Y chromosomal breakpoints of Y(+)XX males should provide insights into the underlying recombination process.

We have previously localized a putative Y-chromosomal breakpoint in a Y(+)XX male to a 35 kb region (Vergnaud et al., 1986). The present paper reports the analysis of this breakpoint. The rearrangement occurred between a Y-chromosomal locus and a pseudoautosomal locus via homologous recombination between repetitive Alu elements (Schmid and Jelinek, 1982). The recombination point is located in the putative RNA polymerase III promoter region found within these repeated sequences (Elder et al., 1981). The present results are discussed in relation to similar findings observed in cases of familial hypercholesterolemia (reviewed in Lehrman et al., 1987a, 1987b).
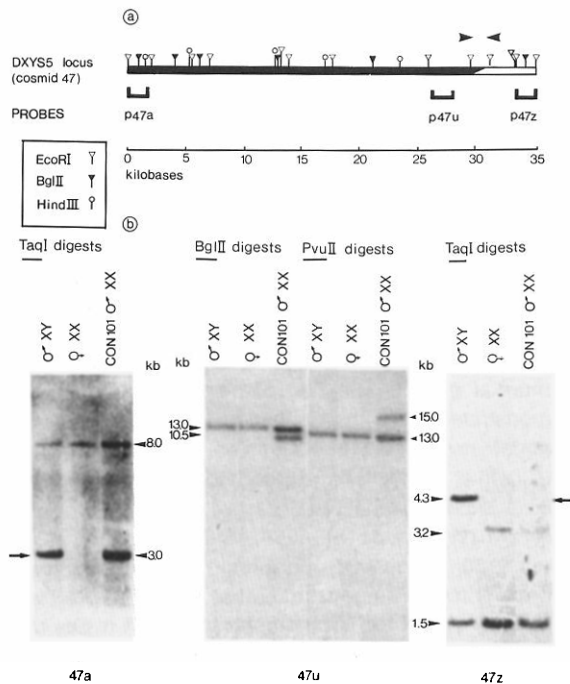
Figure 1. Detection of the Rearrangement at Locus *DXYS5* in CON101 DNA

(a) Restriction map of cosmid 47, derived from Y-chromosomal locus *DXYS5*. The black bar represents sequences present in CON101 DNA, and the open bar those not detected in this XX male. Arrows indicate the approximate position of the breakpoint. Probes 47a and 47z were described in Vergnaud et al. (1986). Probe 47u is a 2.5 kb EcoRI–Sau3A DNA fragment subcloned in pBR327.

(b) Hybridization of probes 47a, 47u, and 47z to Southern blots of 46,XY male, 46,XX female, and CON101 46,XX male DNAs. Arrows indicate the male-specific bands. Blots were washed at 68°C in 0.1× SSC for probes 47a and 47z and in 0.5× SSC for probe 47u.

Results

A Rearrangement in the Most Proximal Yp DNA Sequences in a Human XX Male

We have previously reported an XX male (CON101) in whom the Y-specific DNA sequence detected by probe 47a is present, while other Y-specific sequences present in other XX males studied were absent (Vergnaud et al., 1986). Probe 47a (Figure 1a) derives from one end of the insert of cosmid 47 (Bishop et al., 1983, 1984). Probes isolated from cosmid 47 define locus *DXYS5* (see Goodfellow et al., 1985), which consists essentially of highly homologous sequences located on both the Y chromosome short arm (Vergnaud et al., 1986) and the X chromosome long arm (Geldwerth et al., 1985). Probe 47z, which originates from the other end of the cosmid 47 insert, does not detect any Y-specific sequences in CON101 (Figures 1a and 1b). These results suggested that the Y-chromosomal breakpoint in this XX male is found between sequences corresponding to probes 47a and 47z. Consistent with this model, no Y-specific DNA sequence proximal to *DXYS5* has been detected in this patient, while several Y-specific
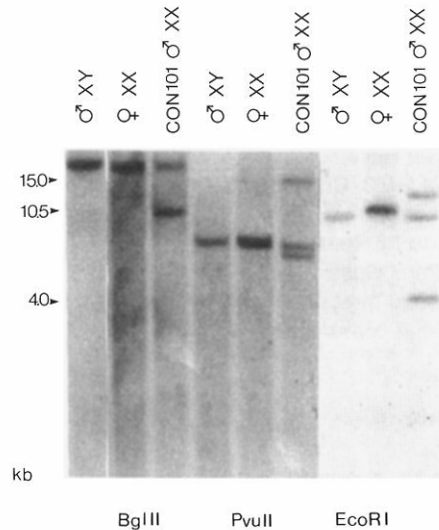

Figure 2. Hybridization Pattern of Probe U7A (Locus *U7*)

Probe U7A is a 250 bp NarI–AccI DNA insert from λ UBgl (see Figure 6). The sizes of the X–Y junction fragments detected in CON101 are shown. Washes were at 68°C in 1× SSC.

sequences distal to *DXYS5* are present (D. C. P., unpublished results).

To analyze this rearrangement at *DXYS5* further, a total Sau3A digest of cosmid 47 DNA was subcloned in pBR327. The inserts of several subclones without repetitive DNA were hybridized to TaqI digests of genomic DNAs from CON101 and normal male and female controls. Three types of homologous fragments were observed in male and female controls: male-specific bands, bands twice as intense in females as in males, and bands of equal intensity in males and females. These homologous fragments are, respectively, specific to the Y chromosome, specific to the X chromosome, and common to the X and Y chromosomes. In numerous instances, bands common to the X and Y chromosomes gave an increased hybridization signal in CON101, suggesting the presence of three copies instead of two. The probes detecting either a Y-specific band or an increased signal in CON101 derive from a 30 kb region at one end of cosmid 47, while all the other probes derive from a 5 kb region at the other end of cosmid 47. Probe 47u, located near the junction of these two clusters, detects a Y-specific band in TaqI-digested CON101 DNA. When hybridized to genomic DNAs digested with BglII or PvuII, 47u detects only a fragment common to the X and Y chromosomes in normal males and females, but it also detects an additional band in CON101 (Figure 1b). The 10.5 kb BglII and 15 kb PvuII fragments seen in CON101 with probe 47u (Figure 1b) were not found in any of 10 normal males and females examined (data not shown). Since these unusual bands were observed in CON101 with different restriction digests, they could span his Y-chromosomal breakpoint. Restriction mapping and hybridization patterns of the various probes from cosmid 47 assigned the putative breakpoint to an interval of 1 kb (arrows in Figure 1a).

DNA Sequences Located beyond the Breakpoint Are Pseudoautosomal

To analyze this putative breakpoint in CON101, the unusual 10.5 kb BglII fragment was cloned in λ 47.1. Recombinant phage clones were screened with probes 47u and 47z. Clones positive with 47u and negative with 47z were purified. (Clones hybridizing with both probes most likely derive from the homologous region on Xq.) One clone, UBgl, was analyzed by restriction mapping. Nine kilobases from one end of its insert was identical to the cosmid 47 insert, whereas the remaining 1.5 kb was not. Based on a fine restriction map of this region, we isolated probe U7A, a 250 bp DNA fragment that is free of repetitive sequences and that appears to lie beyond the Y-chromosomal breakpoint. Probe U7A was hybridized to genomic DNA blots from CON101 and male and female controls. As expected, probe U7A, like probe 47u, detected the 10.5 kb BglII and 15 kb PvuII fragments in CON101 DNA (Figure 2). Fortunately, probe U7A detects diallelic restriction fragment length polymorphisms (RFLPs) with some digests. In numerous male and female individuals, U7A hybridizes to one or two DNA fragments at 6 and 7.5 kb with BamHI digests and at 6.7 and 8.2 kb with EcoRI digests (data not shown). These RFLPs are caused by a 1.5 kb insertion-deletion that encompasses a PvuII site, and generates a PvuII RFLP with a 0.6 kb size variation (6.9 and 7.5 kb fragments). As shown in Figure 2, CON101 displays a diallelic pattern with EcoRI and PvuII digests, whereas the two other individuals are homozygous for the locus U7. Moreover, in CON101, probe U7A detects a 4 kb EcoRI fragment (Figure 2) and a 2.1 kb BamHI fragment (not shown); these fragments have never been observed in normal individuals (Figure 2 and not shown). These displaced bands were assigned to the CON101 breakpoint.

To determine the chromosomal location of locus U7 defined by probe U7A, DNAs from human–rodent hybrid cell lines HORL.9X (Figure 3) and 853-CHO (not shown)—containing the X and Y chromosomes, respectively, as the only human chromosomes—were probed with U7A. Probe U7A detected the normal hybridization band of 1 kb in TaqI digests of both hybrids; U7A did not hybridize to control rodent DNA. This signal was amplified in OX DNA, a human cell line with a 49,XYYYY karyotype (Figure 3). These results assign locus U7 to both the X and Y chromosomes. Regional localization was carried out using a panel of partial human X chromosomes segregated on rodent backgrounds. Probe U7A detects a 1 kb TaqI fragment in hybrid CerCH-S, which contains the chromosomal segment Xpter-q11. No hybridization signal is observed with hybrids CerCH-H and A9-HRBC2, which lack, respectively, the entire X short arm and the region Xp22-pter (Figure 3). These data localize the X and Y chromosome common locus U7 to Xp22, the most distal band on Xp. Furthermore, the case of the two allele RFLP detected by U7A, homozygotes for either allele occur among normal (46,XY) males (data not shown). Taken together, these results suggest a pseudoautosomal location for locus U7.

The sex linkage of locus U7 was therefore tested by family analysis. In male meiosis, the locus U7 segregates in-
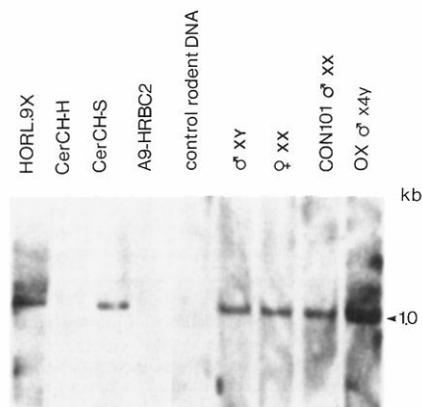

Figure 3. Chromosomal Assignment and Regional Localization of Locus U7

Hybridization of probe U7A to TaqI digests. The human lymphoblastoid cell line OX is derived from a 49,XYYYY patient (see Bishop et al., 1983). The somatic rodent hybrid cell lines and their human chromosomal content are as follows: HORL.9X, entire X (Goodfellow et al., 1980); CerCH-H, Xq11-Xqter; CerCH-S, Xpter-Xq11; A9-HRBC2, Xp22-Xqter (Oberlé et al., 1986).

dependently of sex phenotype in 13 of 35 meioses (37% recombination). This partial sex linkage demonstrates the pseudoautosomal location of U7.

Genetic mapping of U7 relative to five reference pseudoautosomal loci was realized by three-point analyses (Table 1). The genetic map of these five pseudoautosomal loci relative to TDF, and their recombination frequencies relative to sex phenotype, are as follows:

centromere–TDF–MIC2–DXYS17–DXYS15–DXYS28–DXYS14.
(2%) (11%) (35%) (38%) (50%)

This map is drawn from Rouyer et al. (1986b) for MIC2, DXYS17, DXYS15, and DXYS14, and from Page et al. (1987b) for DXYS28. The possible location of U7 in each of these intervals was tested, and the results are reported in Table 1. If U7 belongs to one of the intervals TDF–MIC2, MIC2–DXYS17, DXYS17–DXYS15, or DXYS15–DXYS28, the double recombinations required to account for the observed segregation would be 11/25, 10/25, 6/35, and 1/6, respectively (recombination of loci A + B with U7; see Table 1). Similarly, 1/6 double recombinations would be necessary to place U7 distal to DXYS14 (recombination of locus B with A + U7; see Table 1). Each of these locations involves double recombinations never observed until now in the human pseudoautosomal region (Rouyer et al., 1986a, 1986b; Goodfellow et al., 1986; Page et al., 1987b). In contrast, no double recombinations are required to locate U7 between the distal pseudoautosomal loci DXYS28 (38% recombination with sex) and DXYS14 (50% recombination with sex). It follows that the order of the loci is TDF–MIC2–DXYS17–DXYS15–DXYS28–U7–DXYS14. Taken together, these results show that a rearrangement in CON101 produced a junction between the Yp-specific locus DXYS5 and the distal pseudoautosomal locus U7.

Table 1. Localization of Locus *U7* by Three-Point Analysis in Male Meiosis

	Reference Loci					
Recombination	Locus A: Locus B:	TDF MIC2	MIC2 DXYS17	DXYS17 DXYS15	DXYS15 DXYS28	DXYS28 DXYS14
Locus A/(B + *U7*)		0	1	5	0	1
Locus B/(A + *U7*)		0	0	0	0	1
(Loci A + B)/*U7*		11	10	6	1	0
Nonrecombinants		14	14	24	5	4
Total meioses		25	25	35	6	6

The loci in the columns for "locus A" are given in their order on the genetic map; see text for details. Family DNA samples were provided by the Centre d'Etude du Polymorphisme Humain. Probes used were pSG1 and 19B (Goodfellow et al., 1986) for locus *MIC2*; 601 (Rouyer et al., 1986a) for locus *DXYS17*; 113D (Simmler et al., 1985) for locus *DXYS15*; pDP411a (Page et al., 1987b) for locus *DXYS28*; and 29C1 (Cooke et al., 1985) for locus *DXYS14*. Probe U7A (locus *U7*) was hybridized on EcoRI digests.

A Different Dosage of the Proximal and Distal Pseudoautosomal Loci

That Y(+)XX males are the result of terminal X–Y interchange has recently been verified (Petit et al., 1987; Page et al., 1987a); this mechanism likely accounts for XX male CON101 (see Discussion). According to this model, diagrammed in Figure 4 as it relates to patient CON101, pseudoautosomal sequences proximal to the breakpoint in *U7* should be present in three copies (one each from the paternal X, the paternal Y, and a maternal X chromosome). Those pseudoautosomal loci distal to *U7* would be present in only two copies (one from the paternal Y chromosome and one from a maternal X chromosome).

We therefore determined the copy number of several pseudoautosomal loci in CON101. To obtain a breakpoint-distal probe, a nonrearranged 8.2 kb EcoRI fragment from pseudoautosomal locus *U7* was isolated in the λ gtWES vector as clone U7R. Clone U7R should contain both breakpoint-proximal and -distal DNA sequences, present in three and two copies, respectively, in CON101 DNA. Probe U7A, which hybridizes to both rearranged (UBgl) and normal (U7R) pseudoautosomal fragments, defines the proximal part of U7R (see Figure 6). As observed in Figure 2, it detects three copies of this locus (two normal alleles and one rearranged fragment) in CON101 DNA. In contrast, probe U7T is present in the normal cloned allele U7R, but is missing in the recombined fragment UBgl (see Figure 6), and corresponds to the distal part of U7R. In EcoRI digestions, this probe recognizes only the two normal alleles in CON101 DNA, as it does in male and female controls (Figure 5A). This result is consistent with the terminal-interchange model, which implies deletion of all pseudoautosomal sequences distal to the breakpoint on the rearranged X chromosome (see Figure 4). Unfortunately, the complex hybridization pattern observed with probes from the telomeric locus *DXYS14* did not permit determination of the copy number in CON101 in the absence of DNA from the patient's parents. Probes from pseudoautosomal loci proximal to the breakpoint have been tested on various CON101 DNA digests. Figure 5B shows the hybridization pattern observed with probes 113D (*DXYS15*) and 601 (*DXYS17*). Three different alleles are observed with 113D, while the hybridization pattern shown by 601 consists of an upper band of normal intensity and a lower
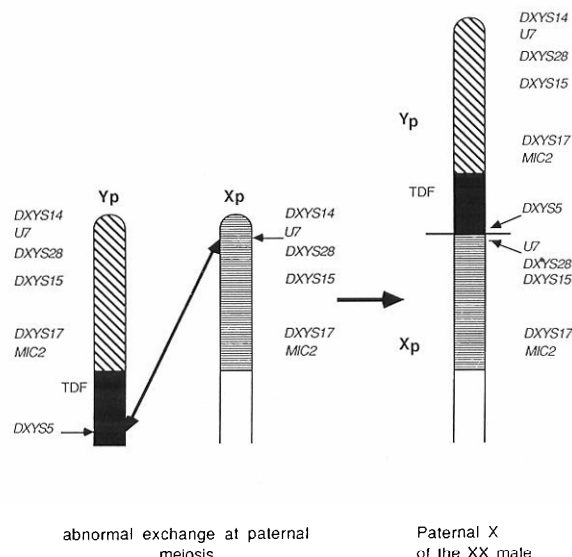

Figure 4. Schematic Representation of the Abnormal X–Y Interchange

Sex-linked locus *DXYS5* and pseudoautosomal loci *DXYS14, DXYS15, DXYS17, DXYS28,* and *MIC2* are named according to the Human Gene Mapping 8 nomenclature (Goodfellow et al., 1985). Obliquely and horizontally striped boxes represent, respectively, the Y and X paternal pseudoautosomal regions. Black boxes correspond to Y-chromosomal sex-linked sequences, and white regions correspond to X-specific sequences. The breakpoint is shown as a horizontal bar.

band of double the usual intensity. Since the parents of this patient were deceased, the parental origin of the different pseudoautosomal alleles observed in CON101 could not be determined. Normal males and females may be either homozygous or heterozygous at these loci, but we have never observed three copies of either locus in a normal individual (Figure 5B and Simmler et al., 1987). Moreover, ten other XX males have two copies of these loci, as in normal males and females (Figure 5B and not shown).

The Rearrangement Is Caused by Homologous Alu–Alu Recombination

Figure 6 shows the restriction map of the breakpoint region on the normal Y chromosome (cosmid 47), the X–Y
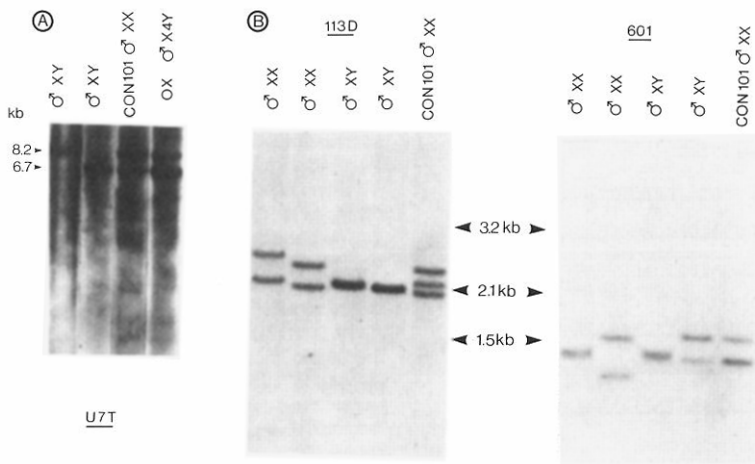
Figure 5. Dosage of the Pseudoautosomal Loci in CON101

(A) Hybridization pattern of probe U7T on EcoRI digests. Probe U7T is a 4.6 kb SmaI DNA fragment from λ U7R (see Figure 6). Hybridization was performed with a large excess of non-radioactive human genomic DNA (according to Litt and White, 1985). Washes were at 68°C in 0.1x SSC.

(B) Hybridization pattern of probes 113D (locus DXYS15) and 601 (locus DXYS17) on TaqI digests. Insert 601 consists of tandemly repeated short elements (Simmler et al., 1987) and thus reacts more strongly with larger alleles of this polymorphic locus (second and fourth lanes for probe 601). For the two probes washes were at 68°C in 2x SSC.

rearrangement from CON101 (phage λ UBgl), and the normal X-pseudoautosomal fragment (phage λ U7R). Orientation relative to the telomeres and centromeres is defined by the model shown in Figure 4. The restriction map of the left part of UBgl (up to the first Alu repeat) is identical to the map of cosmid 47. The right part of UBgl (from this Alu sequence to the end of the insert) is identical to the map of U7R. Thus these results map the breakpoint within an Alu repeat. A more precise localization of the recombination point was deduced from a comparison of the Y, rearranged X-Y, and X-pseudoautosomal nucleotide sequences (Figure 7). Outside the Alu element there is no significant homology between the normal Y (47) and X (U7R) sequences. Sequencing of approximately 200 bp on either side of the Alu sequences on the X and Y chromosomes confirmed the absence of homology already observed by restriction mapping (not shown). In contrast, the Alu elements from clones 47 and U7R are 87% homologous, typical of nonallelic Alu sequences (Deininger et al., 1981). Furthermore, the recombined Alu sequence can

very easily be aligned with the normal counterparts. No major sequence rearrangement has occurred within the Alu element, suggesting that it is the product of homologous Alu–Alu recombination.

Since the three DNA clones derive from three unrelated individuals, mismatches observed in both the Y (47 versus UBgl) and the X (U7R versus UBgl) portions of the junction (Figure 7) are probably due to polymorphic variations. The difference between such alleles is much less than the difference observed between two nonallelic Alu sequences. UBgl and U7R sequences differ by only 3 of 87 bp from the beginning of the Alu repeat up to position −81 (3.4% divergence). Twenty mismatches and a 6 bp deletion are found within 207 bp, from position −80 up to the end of the poly(A) stretch (12.6% divergence). From the poly(A) stretch at the end of the Alu sequence to position −96, UBgl and 47 differ at 3 of 224 bp (1.3% divergence). Beyond this position, the match is less perfect, with 10 of 72 bp differing (13.9% divergence). These sequence comparisons suggest that recombination occurred within a
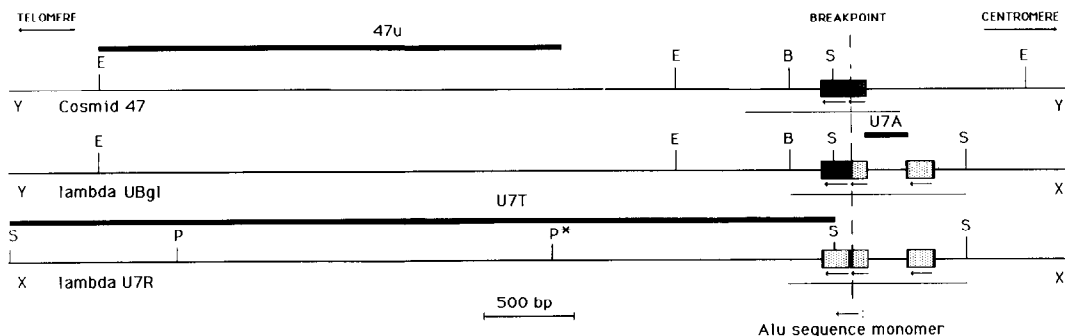

Figure 6. Restriction Maps of the DNA Region Overlapping the X-Y Junction

The top map shows the DXYS5 locus (cosmid 47); the bottom map shows the U7 locus (λ U7R). The middle map represents the rearranged fragment of CON101 (λUBgl). The restriction sites are as follows: E, EcoRI; B, BamHI; S, SmaI; P, PvuII. The horizontal black bars above each map indicate the location of the different probes. The PvuII site with the asterisk (bordering a 2.1 kb PvuII fragment) is lost in the smaller, normal allele of locus U7 recognized by probe U7A. Telomeres and centromeres are designed according to Figure 4. The vertical dashed line represents the breakpoint between the two normal loci. Stippled boxes correspond to Alu sequences from the X (lighter pattern) and Y (darker pattern) chromosomes; vertical black bars represent poly(A) stretches. The orientation of the Alu sequences is according to Deininger et al. (1981) and is indicated by the arrows. Fine lines below the breakpoint regions in the three clones indicate the sequenced DNA fragments.

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<---- Centromere
                                                       <--A BOX->
TAACACAGCCAGGATATTGACATTGATAAGATTCATTGACATGG-GCTGGACTTGGTGGC (47)
***  *********  *** * *** *  *** * ******    *   *  ***
TTGGAGCAGTGACGAAGCCAGACACAGAAAGATAAACACTGTGG-GCCGGGCGCCGTGGC (UBg1)
                                            *
TTGGAGCAGTGACGAAGCCAGACACAGAAAGATAAACACTGTGTGGGGCCGGGCGCCGTGGC_ (U7R)
                                   -177|

                                                            <--
TCACGCCTGTAATCCCAGCACTTTGGGAGGCCAAGGAGGGCGAATCACAAGGTCGGGAGA (47)
                         *    *      *       *        *
TCACGCCTGTAATCCCAGCACTTTGGGAGGCCGAGGCGGGCGGATCACGAGGTCAGGAGA (UBg1)
                                          *       *
TCACGCCTGTAATCCCAGCACTTTGGGAGGCCGAGGCAGGCGGATCATGAGGTCAGGAGA_ (U7R)
                                         -97|~~~~~
-B BOX->
TCGAGACCATCCTGGCTAAGACGGTGAAACCCCGTCTCTACTAAAAATACAAAAAA-TTA (47)

TCGAGACCATCCTGGCTAAGACGGTGAAACCCCGTCTCTACTAAAAATACAAAAAA-TTA (UBg1)
          **        *        *                            *
TCGAGACCATCTCGGCTAACACGGTGAAAGCCCGTCTCTACTAAAAATACAAAAAAATTA (U7R)
~~~~~~~~~~~|-80

GCCGGGCGTGGTGCGGGCGCCTGTAGTCCCAGCTACTCGGGAGGCTGAGGCAGGAGAATG (47)
        *
GCCGGGCGAGGTGCGGGCGCCTGTAGTCCCAGCTACTCGGGAGGCTGAGGCAGGAGAATG (UBg1)
*   * **  *                          *
ACCAGATGTGGTGCGGGCGCCTGTAGTCCCAGCTACTCGGCAGGCTGAGGCAGGAGAATG (U7R)
                        |0

GCGTGAACCCGGGCGGCGGAGCTTGCTGTGAGCCGAGATCACACCACCGCCCTCCAGCCT (47)
                   *
GCGTGAACCCGGGCGGCGGAG-TTGCTGTGAGCCGAGATCACACCACCGCCCTCCAGCCT (UBg1)
       *             *              * *     *  * **
GCGTGAACCCGGGAGGCGGAG-TTGCAGTGAGCCGAGATCGCGCCACTG-ACTCCAGCCT (U7R)


GGGAGACAGA------CTGCATCTCAAAAAAAAAAAAAAAAAGATTCATTGACATTGACTCA (47)
          *
GGGAGACAGA------CTGCATCTCAAAAAAAAAAAAA-AAGATTCATTGACATTGACTCA (UBg1)
 *    ******  *            *       *  * *** *** *** ******
GGGCGACAGAGCAAGACTCCATCTCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAGATACACAC (U7R)
                     |+116
              Telomere ---->
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Figure 7. DNA Sequence of the Breakpoint Region

Sequences from *DXYS5* (cosmid 47, top line), *U7* (λU7R, bottom line) and the rearranged fragment (λUBgl, middle line) are aligned in accordance with the best matching of the Alu repetitive sequences. Orientation with respect to the telomere and centromere is according to the model in Figure 4. The Alu elements are underlined, and the DNA sequences are oriented and numbered (the vertical bars indicate the precise considered nucleotide) according to the consensus sequence of Deininger et al. (1981). Direct repeats flanking the Alu elements are shown in boldface type. Asterisks correspond to nucleotide mismatches. The wavy line indicates the 16 bp segment containing the recombination point. The A and B boxes of the putative RNA polymerase III control region are indicated according to Paollela et al. (1983).

stretch of 16 bp, from position −81 to −96 in the first monomer of the Alu sequence.

Discussion

X–Y Rearrangement

This study reports the molecular analysis of a chromosomal breakpoint in a human Y(+)XX male, occurring between Yp-specific locus *DXYS5* and pseudoautosomal locus *U7*. It has been shown that human Y(+)XX maleness results from an interchange of terminal parts of the X and Y chromosome short arms (Petit et al., 1987; Page et al., 1987a). In this abnormal interchange, the distal part of the Yp, including TDF, is transferred to the terminal part of Xp. In most cases there is complete loss of the paternal X pseudoautosomal region. In some cases this Xp deletion extends to X-specific sequences located in Xp22.3 (Petit et al., 1987). However, the point of recombination on the X chromosome can also be located in the pseudoautosomal region (Page et al., 1987a). If the XX male CON101 is also the result of an X–Y interchange, then the exchange occurred between *DXYS5* on the Y chromosome and pseudoautosomal locus *U7* on the X chromosome. Since this hypothesis cannot be fully verified in the absence of the parental DNAs, alternative possibilities must be discussed.

The presence in CON101 of an autonomously segregat-

ing Y-chromosomal fragment is unlikely, given absence of the Y centromere (Vergnaud et al., 1986). Alternatively, the *DXYS5–U7* rearrangement may preexist in the paternal Y chromosome. In that case, the rearrangement might be transmitted by the proband via X–Y interchange, if compatible with fertility. This rearrangement could consist either of a duplication or an inversion. Recombination between the *DXYS5* and *U7* loci on Yp sister chromatids could have generated the observed breakpoint. The newly rearranged Y chromosome would show an internal duplication of the Yp region between *DXYS5* and *U7*, including TDF. Subsequently, a normal meiotic X–Y crossover occurring in the internally duplicated part of the pseudoautosomal region would generate XX males. Such a situation would be reminiscent of the sex-reversing mutation in the mouse (Singh and Jones, 1982; Evans et al., 1982) and could account for some familial cases of Y(+)XX maleness. Similarly, an internal Yp inversion between the *DXYS5* and *U7* loci could preexist in the father's Y chromosome and account for the breakpoint. Inversions involving Yp-specific sequences have been proposed to explain abnormal patterns of Yp sequences observed in some XX males and XY females (Affara et al., 1986; Disteche et al., 1986; Page, 1986). This abnormal Y chromosome may then undergo an interchange with the X chromosome, mobilizing TDF. However, in the case of CON101 the breakpoints caused by the interchange would have to be

located in the vicinity of the *U7* locus on each paternal sex chromosome. It appears improbable that three abnormal events occur in this limited region.

As mentioned above, familial XX maleness may sometimes be ascribed to Y rearrangements preexisting in the father. But familial XX maleness is rather infrequent, and, to date, Y-specific DNA has been detected in XX males from only one such family (Page et al., 1985). Duplication of pseudoautosomal sequences in fathers of sporadic XX males has also not been reported. Conversely, a terminal X-Y interchange giving rise to an X-chromosomal breakpoint within the pseudoautosomal region has been reported recently for an XX male (case LGL1358 in Page et al., 1987a). Therefore, terminal X-Y interchange is most likely the cause of the *DXYS5–U7* rearrangement observed in XX male CON101. Moreover, the X-Y interchange model implies the existence of two reciprocal products generated by the recombination event. 46,XY women with a deletion of distal Yp-specific sequences have been reported (Disteche et al., 1986), and the loss of proximal but not distal paternal pseudoautosomal loci has also been observed in such cases (Vergnaud et al., unpublished). These XY females could thus represent the reciprocal product of the X-Y interchange observed in XX male LGL1358, and support the existence of interchanges between the X-pseudoautosomal region and Yp-specific sequences.

Alu–Alu Homologous Recombination

DNA sequence analysis of the chromosomal breakpoint shows that homologous recombination has taken place between two Alu repeated elements that are 87% homologous, a degree of homology typical of nonallelic Alu sequences. Sequence divergence is much greater between nonallelic than allelic repeats—for example, two alleles (in UBgl and U7R) of the Alu monomeric element on the right side of the breakpoint in locus *U7* (see Figure 6) differ at only one of 142 nucleotides. Accordingly, it appears that the recombination occurred somewhere between positions −96 and −81. At each side of this breakpoint region, there remain three nucleotide differences between the two allelic forms sequenced. These differences probably reflect allelic variation.

Homologous recombination between Alu sequences in introns of the low density lipoprotein (LDL) receptor gene has been reported in three patients with familial hypercholesterolemia (Hobbs et al., 1986; Lehrman et al., 1987a, 1987b). To date, 17 breakpoints caused by homologous or nonhomologous recombination at Alu sequences have been described either within the LDL receptor gene or in the α- and β-globin gene clusters. As in the present case, 12 of these 17 breakpoints occurred in the first monomer of the Alu element (Lehrman et al., 1987b; Nicholls et al., 1987). In vitro transcription of some Alu sequences by RNA polymerase III initiates in this first monomer (Elder et al., 1981). RNA polymerase III transcription is controlled by an internal split promoter in tRNA genes (Hofstetter et al., 1981). This promoter is also found in transcribed Alu repeats (Elder et al., 1981; Paollela et al.,

1983). Among the 12 breakpoints localized in the first Alu monomer, 10 occurred between the A and B boxes of this putative RNA polymerase III internal promoter. In CON101, recombination took place in a 16 bp segment that overlaps an 11 bp sequence (AGATCGAGACC) sharing homology with the consensus sequence GGTTCGANNCC of the promoter box B.

It has been suggested that a transcription-induced DNA deformation precipitates recombination events in this part of the Alu sequence (Lehrman et al., 1987a, 1987b). Transcriptional stimulation of recombination has been demonstrated in yeast mating-type switching (Klar et al., 1981) and in V-region gene rearrangements in cultured, transformed precursor B lymphocytes (Blackwell et al., 1986). Recently, stimulation of genetic exchange by sequences regulating RNA polymerase I transcription was demonstrated in yeast (Voelkel-Meiman et al., 1987). In this latter study, stimulation of recombination required transcription of both copies of the homologous recombining sequences. If actual transcription of Alu sequences is necessary for recombination, one might expect to find a scattering of breakpoints along the length of the dimer. However, almost all breakpoints analyzed are clustered in the RNA polymerase III promoter region. Perhaps recombination is stimulated simply by the binding of the transcription complex to this control region. In either case, these studies suggest a role for transcriptionally active Alu sequences in genetic exchanges.

The homologous recombination events analyzed in the LDL receptor gene occur in autosomes and therefore do not provide any clue as to their meiotic (between homologous chromosomes) or mitotic (between sister chromatids) origin. Although a mitotic event occurring in the paternal germ line cannot be totally ruled out, the present study suggests that Alu–Alu recombination can take place at meiotic prophase I, during X-Y pairing. In addition, the present homologous recombination event between two nonhomologous loci could be viewed as an abnormal crossing over occurring instead of the normal obligatory one observed between the human X and Y chromosomes. If abnormal crossing over requires homologous recombination, Alu repeated elements may be used because of their widespread occurrence throughout the genome. More generally, Alu–Alu recombination could be a phenomenon involved in other abnormal chromosomal rearrangements, such as translocations and inversions.

Experimental Procedures

DNA Extractions and Southern Blot Analysis

Genomic Cloning

CON101 DNA was digested with an excess of BglII and fractionated by centrifugation through a 10%–40% sucrose gradient. The 9–12 kb fraction, corresponding to the hybridization band detected by probe 47u, was precipitated and redissolved in Tris- EDTA buffer, and an aliquot of 100 ng was ligated with λ 47.1 BamHI arms for 16 hr at 14°C. The DNA was packaged in vitro (Gigapack, Stratagene), and about 5 × 10^5 phage were plated on Escherichia coli strain LA101. Five positive clones were obtained by transferring plaques to filters (Hybond-N) and screening them with probe 47u. One was also reactive with probe 47z, which hybridizes to the same BglII fragment located on the homologous region Xq13-q24, and discarded. Among the four other clones, positive with 47u but not with 47z, one (UBgl) was further analyzed. The restriction map of clone UBgl was consistent with the sizes of restriction fragments observed on Southern blots of CON101 DNA. U7R was cloned in a similar manner from a lymphoblastoid cell line of a 49,XYYYY man. DNA was digested with EcoRI, and the appropriate fraction was ligated with λ gtWES EcoRI arms (BRL). After in vitro packaging, 4 × 10^5 plaques were obtained, transferred onto filters, and screened with probe U7A. One positive clone, U7R, was isolated, and the insert was subcloned in the Bluescribe vector (Stratagene). Subsequent restriction mapping was in accordance with the Southern hybridization results.

Sequencing Procedures

DNA sequences were determined by the dideoxynucleotide chain termination method (Sanger et al., 1977). On cosmid 47, sequencing was carried out on a PstI–Sau3A DNA fragment (800 bp) encompassing the Alu element (see Figure 6). DNA was sonicated, blunt-ended by filling-in with T4 DNA polymerase, and subcloned in a SmaI-cut M13mp8 vector. The corresponding region of UBgl was analyzed from a 700 bp SmaI fragment containing probe U7A, and the adjacent 450 bp SmaI–PstI segment at the telomeric side (see Figure 6), cloned in mp8 and 9 vectors. These inserts were sequenced both directly and after generation of 5′ deletions between restriction sites of the insert and the cloning polylinker. Using this method, sequences of the same 700 bp SmaI fragment and the following 250 bp SmaI–PstI fragment from clone U7R were determined.

Acknowledgments

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