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A DNA fragment from the human X chromosome short arm which detects a partially homologous sequence on the Y chromosomes long arm

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ABSTRACT

An X linked human DNA fragment (named DXS31) which detects partially homologous sequences on the Y chromosome has been isolated. Regional localisation of the two sex linked sequences was determined using a panel of rodent-human somatic cell hybrids. The X specific sequence is located at the tip of the short arm (Xp22.3-pter), i.e. within or close to the region which pairs with the Y chromosome short arm at meiosis. However the Y specific sequence is located in the heterochromatic region of the long arm (Yq11-qter) and lies outside from the pairing region. DNAs from several XX male subjects were probed with DXS31 and in all cases a double dose of the X linked fragment was found, and the Y specific fragment was absent. DXS31 detects in chimpanzee a male-female differential pattern identical to that found in man. However results obtained in a more distantly related species, the brown lemur, suggest that the sequences detected by DXS31 in this species might be autosomally coded. The features observed with these X-Y related sequences do not fit with that expected from current hypothesises of homology between the pairing regions of the two sex chromosomes, nor with the pattern observed with other X-Y homologous sequences recently characterized. Our results suggest also that the rule of conservation of X linkage in mammals might not apply to sequences present on the tip of the X chromosome short arm, in bearing with the controversial issue of steroid sulfatase localisation in mouse.

INTRODUCTION

The X and Y chromosomes in mammals are thought to have originated from a common ancestor. Although these two chromosomes in human are very different with respect to size and gene content, with the Y chromosome highly specialized in sex determination, sequence homologies between the two sex chromosomes might exist as functionally important remnants of this evolutionary history (1). It has been suggested that such homologous sequences should predominantly exist in the segments of the two chromosomes that pair at meiosis : i.e. the distal part of the short arm of the X chromosome and most of the short arm of the Y chromosome (2, 3). In particular, the existence in the pairing region of the X chromosome of two loci (corresponding

to the Xg blood group and to steroïd sulfatase) which escape inactivation (4, 5) has suggested that they are located in a region of functional homology with the Y chromosome, with thus no need for dosage compensation. Support for this hypothesis has come from the demonstration that a gene controlling the expression of a surface antigen in human cells, located at the tip of the short arm of the X chromosome has a functional homolog in the euchromatic region of the Y chromosome (6). The isolation and characterization of DNA sequences from the human X or Y chromosomes should allow to test current hypothesises concerning the regions of homology.

We report here the isolation from the human X chromosome library of Davies et al. (7) of a sequence located in the distal part of the short arm of the X chromosome which detects an homologous but non identical sequence on the Y chromosome, outside of the pairing region. The X linked fragment has been used to probe the genome of subjects presenting anomalies of sex determination. Related sequences which behave like autosomal (or pseudoautosomal) sequences are present in the genome of lemurs.

MATERIALS AND METHODS

Preparation of DNA and Blot hybridization.

DNA preparation, from cultured cell lines or from total leukocytes, digestion with restriction enzymes, blotting onto diazobenzyloxymethyl (DBM)-paper (8) and hybridization (in the presence of 4% dextran sulfate and 40% formamide at 42°C) were as described (9).

Cell lines and DNAs.

Lymphoblastoïd cell lines with a 48 XXXX (GM 1416) and a 49 XXXXY (GM 1202) karyotype were obtained from the Human Genetic Mutant Repository (Camden, New Jersey). A lymphoblastoïd cell line with 49 XYYYY karyotype (10) was obtained from Dr. Fellous (Institut Pasteur, Paris). Mouse-human hybrids derived from the human parents GM 194 (X,3) (q28,q21), GM 97 (X,1) (q26,q12), Anly (X,9) (q12,p24), GO (X,14) (p11,q32), 58 (X,14) (q13,q32) and PI (X,15) (p22,q22) were obtained from Dr. K.H. Grzeschik (Institut für Human Genetik, Munster) and have been previously described (11-14). The hybrid line derived from GM 89 (X,19) (q22,q13) and the HRBC2 A9 hybrid line (12) were obtained from Dr. M. Siniscalco (Sloan Kettering Institute, New York). DNA from the AMIR 2N clone (6) was given by Dr. P. Goodfellow (Imperial Cancer Research Fund, London). Chinese hamster-human hybrid lines derived from human parent 34 (X,2) (p22.3,q32.3) (15) and Cer (X,22) (q11,-q11.2) (16) were obtained from Drs. D. Weil and M.C. Hors Cayla (Inserm U12,

Paris). Cer-CH S is a clone back selected in the presence of azaguanine (16).

The 4 XX male DNAs analysed were those used with other Y chromosome specific probes (17). The DNAs (including DNAs from two XX hermaphrodites) or the blood samples were obtained from Dr. M. Fellous and J. Weissenbach (Institut Pasteur, Paris) or from Dr. G. André (Faculté de Médecine, Strasbourg). DNA from chimpanzees was a gift from Dr. J. Weissenbach. Blood from brown lemurs (lemur fulvus mayottensis) was donated by Dr. Y. Rumpler (Faculty of Medicine, Strasbourg).

RESULTS

An X linked probe that detects Y specific sequences.

As part of a systematic search for genetic markers on the human X chromosome, we are isolating DNA sequences from the genomic library which was constructed by Davies et al., using DNA from purified X chromosome (7). In a first screening, we picked several phages at random and their insert was characterized with respect to size and presence of human repetitive sequences by hybridization to total nick translated human DNA. The 7.5 kb EcoRI insert present in one of the phages was cut by PstI generating a 2.1 kb fragment corresponding to a unique sequence, and a 5.4 kb fragment containing repetitive sequences. The 2.1 kb EcoRI-PstI fragment was subcloned in the plasmid PBR322. In order to determine whether the sequence is coded by the X chromosome, it was hybridized to genomic DNAs from normal males (XY), normal females (XX) and from cells with a 48 XXXX, 49 XXXXY and 49 XYYYY karyotype. These DNAs were first digested with EcoRI and transfered onto diazobenzyloxymethyl (DBM) paper by the Southern blotting technique (8, 9). After the hybridization reaction, the filter was washed under conditions of medium stringency (1 X SSC, 60°C). Autoradiography revealed the presence of a 7.5 kb EcoRI fragment which showed in the various tracks the dosage effect expected for an X linked sequence (Fig. 1). However two additional fragments of 4 kb and 0.7 kb were seen only in the 46 XY, 49 XXXXY or XYYYY samples and the dosage seen in the latter demonstrated that the two fragments are located on the Y chromosome. There was no evidence for the presence of autosomally coded sequences related to the probe. When the same DNA blot was washed under higher stringency conditions (0,2 X SSC, 65°C) the Y specific bands disappeared. The cloned X linked sequence has been denominated DXS31 in the catalogue established at the 7th Human Gene Mapping Workshop (18).

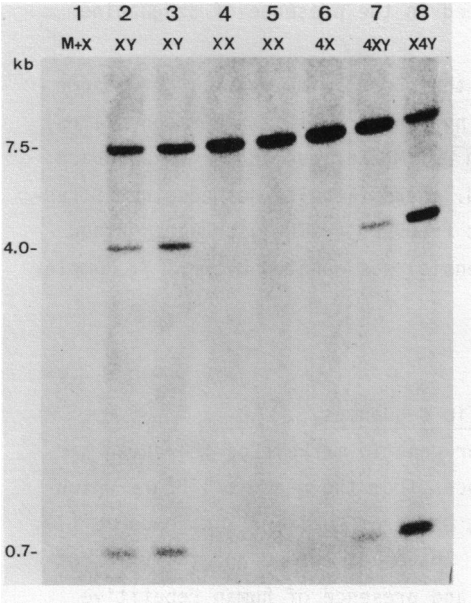
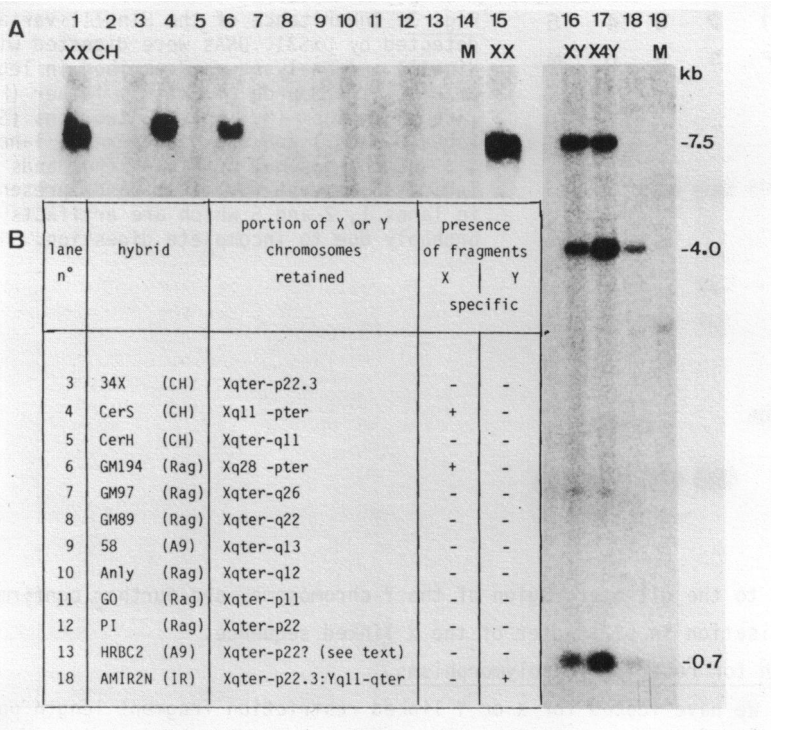


Fig. 1. Dosage analysis of genomic DNA fragments hybridizing to DXS31. DNA samples (15 μg) were digested with EcoRI, electrophoresed on a 0.9% agarose gel, and blotted onto DBM paper (8). The filter was hybridized to the nick translated insert of DXS31 clone at 42°C in 40% formamide. The blot was washed at 60°C in 1xSSC, 0.1% SDS. Source of DNAs : lane 1, hybrid line A9 HRBC2 (M+X, see text) ; lanes 2 and 3, unrelated males (XY) ; lanes 4 and 5, unrelated females (XX) ; lanes 6, 7 and 8, lymphoblastoïd cell lines with a 48 XXXX, 49 XXXXY and 49 XYYYY karyotype, respectively.

Regional localisation on the X and Y chromosomes.

In order to locate sequences on the X chromosome, we use a panel of rodent-human hybrid cell lines derived from human parental cells carrying X-autosome translocations with various breakpoints on the X chromosome. The DNAs from the hybrid cells were digested with EcoRI, tranferred onto DBM paper and hybridized to the 2.1 kb EcoRI-PstI fragment. As shown in figure 2 an hybridization signal corresponding to the X linked EcoRI fragment was seen only with the human DNA controls, the RAG-GM 194 hybrid which contains all the X chromosome sequences from pter to a breakpoint in q28 distal to the G6PD locus (11) and the Cer-CH S hybrid (Xq11-pter) (16). All other cell lines were negative including the Rag-GO and C-34X lines which contain most of the human chromosome from p22 or p22.3 respectively to qter (13, 15). Thus the DXS31 sequence should be located in the p22.3-pter region of the X chromosome. It is worth noting that there is no signal in the HRBC2 hybrid, which was supposed to contain the whole human X chromosome (12). This hybrid however contains all of the 13 other X linked sequences that we have analysed and which are located within p22-qter (to be published). This suggests that a terminal deletion of the short arm of the X chromosome has occured in the HRBC2 line.

In order to locate the Y linked cross-hybridizing fragment we used another hybrid cell line derived from a patient with a (X,Y) (p22.3-q11)



lane n°	hybrid		portion of X or Y chromosomes retained	presence of fragments X specific	presence of fragments Y specific
3	34X	(CH)	Xqter-p22.3	-	-
4	CerS	(CH)	Xq11 -pter	+	-
5	CerH	(CH)	Xqter-q11	-	-
6	GM194	(Rag)	Xq28 -pter	+	-
7	GM97	(Rag)	Xqter-q26	-	-
8	GM89	(Rag)	Xqter-q22	-	-
9	58	(A9)	Xqter-q13	-	-
10	Anly	(Rag)	Xqter-q12	-	-
11	GO	(Rag)	Xqter-p11	-	-
12	PI	(Rag)	Xqter-p22	-	-
13	HRBC2	(A9)	Xqter-p22? (see text)	-	-
18	AMIR2N	(IR)	Xqter-p22.3:Yq11-qter	-	+

Fig. 2. Regional localization of DNA fragments hybridizing to DXS31 on the X and Y chromosome. A : DNAs were digested and analysed as described in Fig. 1. Source of DNAs : lanes 1 and 15, normal human female (XX) , lane 16 normal male (XY) ; lane 17, lymphoblastoïd cell line from a 49 XYYYY male (X4Y) ; lane 2, chinese hamster cells (CH) ; lanes 14 and 19, mouse L cells (M) ; lanes 3 to 13 and 18, human-rodent hybrid cell lines described in panel B : the type of rodent cells used to construct the various hybrids is indicated in parenthesis.

translocation (19). The AMIR-2N clone has been selected successively in HAT medium and for the absence of expression of the 12 E7 antigen (6) and contains as the only sex chromosome the derivative chromosome (Xqter-p22.3 : Yq11-qter). DNA from this clone was analysed by Southern blotting with the DXS31 probe. This showed that the X linked EcoRI fragment was absent, while the two Y linked EcoRI fragments were present (Fig. 2, lane 18). The ratio of the intensity of signal in the AMIR-2N clone and in male DNA was the same as that found for probes localised in the X p22.3-qter region of the derivative chromosome (results not shown), establishing that the presence of the Y linked EcoRI fragments in the hybrid line correlates well with the presence of the translocation chromosome. These results assign the Y linked frag-

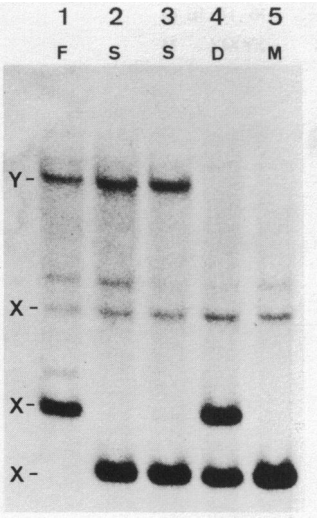


Fig. 3. Inheritance of the HindIII variant detected by DXS31. DNAs were digested with HindIII and analysed as described in legend of Fig. 1. Source of DNAs : father (F, lane 1), mother (M, lane 5), two sons (S, lanes 2 and 3) and one daughter (D, lane 4). The chromosomal origin of the bands is indicated, except for minor bands present in lanes 1, 2 and 5 which are artifacts probably due to incomplete digestion.

ments to the q11-qter region of the Y chromosome, and further confirm the localisation in p22.3-qter of the X linked sequence.

Search for restriction polymorphisms.

We have looked for X or Y linked restriction fragment length polymorphism (RFLP) detected by the DXS31 probe, using 10 restriction enzymes to digest DNAs from 5 to 15 individuals. Male specific sequences were detected under hybridization and washing conditions of medium stringency, with the enzymes TaqI, MspI, PvuII, HindIII, BglII. Except for the sex linked differences the only indication for existence of a RFLP was found with HindIII. In a first screening a female was found having two fragments of 2.8 kb and 2.4 kb while in other females DNAs only the 2.4kb fragment was present. Analysis of the family fitted with an X-linked inheritance of these fragments (Fig. 3). However out of 54 independent X chromosomes tested, we did not found any other occurence of the 2.8 kb band. It corresponds thus to a rare variant in the caucasian population tested and is not useful at present in genetic analysis.

Analysis of the genome of XX males and true hermaphrodites.

We have analysed the DNAs from 4 XX males and 2 XX true hermaphrodites using the DXS31 probe. Parallel studies have shown that the genome of three of these XX males contain different but overlapping parts of the Y chromosome (cases 1, 3 and 4 in ref 17). In contrast we did not find any signal corresponding to the Y linked fragments in all 6 DNAs, while the dosage of

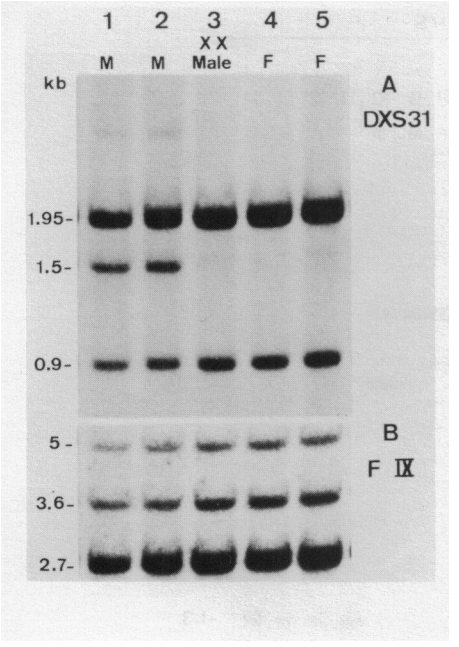


Fig. 4. Inheritance of DXS31 in the family of an XX male. DNAs were digested with TaqI and analysed as described in Fig. 1. Source of DNAs : lane 1, normal male ; lane 2, father of the XX male ; lane 3, XX male (case 4 in ref 17) ; lane 4, mother of the XX male ; lane 5, unrelated female.
A. Hybridization with DXS31 probe.
B. Hybridization of the same blot with an other X linked sequence (the cDNA for coagulation factor IX) shown (in part) as a control for dosage comparison.

the X linked fragments was the same as for normal females (for case 4, see Fig. 4, other results not shown).

DXS31 related sequences in chimpanzees and lemurs.

Analysis of evolutionary conservation might give useful information concerning the origin and potential importance of such sequences of unknown function. We analysed first DNAs from a male and a female chimpanzee. In EcoRI digests the pattern obtained was identical to that found in humans (Fig. 5, lanes 1 to 4). This suggests that the sequences detected by DXS31 are present in the X and Y chromosomes of chimpanzee as they are in humans. A completely different pattern was obtained with DNA from a much more distantly related prosimian species, the brown lemur. The DXS31 probe detected several fragments in DNA from two male and two female lemurs digested with EcoRI, PvuII or TaqI (Fig. 5, lanes 5 to 14). The bands showed neither a gene dosage indicative of X linkage nor a male specific pattern. In contrast, hybridization of the same blot with a cDNA probe for the X linked human coagulation factor IX showed very clearly the dosage expected for X linked fragments (results not shown). Furthermore in TaqI digests the DXS31 probe detected individual variations suggesting restriction site polymorphisms : a strong band of 4.4 kb was present with an identical dosage in one

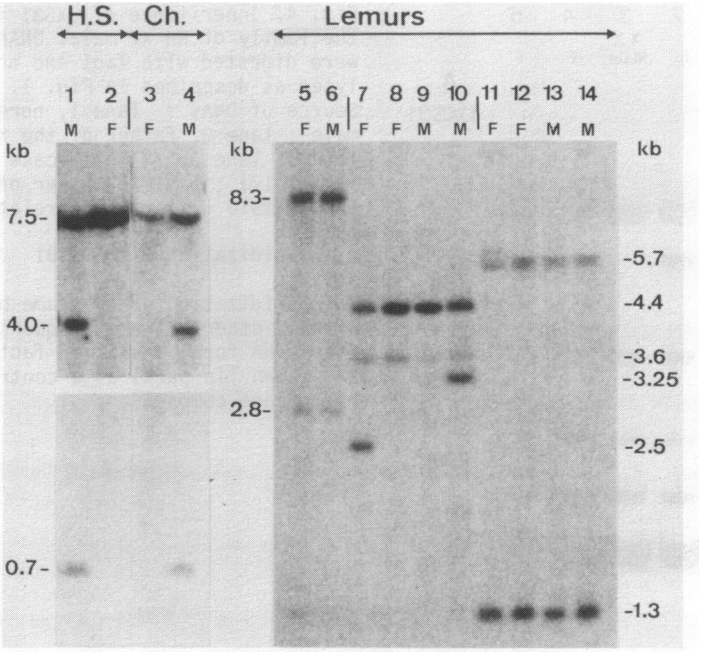


Fig. 5. Conservation of sequences homologous tc DXS31 in chimpanzee and lemurs. DNAs were digested with EcoRI (lanes 1 to 6), TaqI (lanes 7 to 10) and PvuII (lanes 11 to 14). Blotting and hybridization were as in Fig. 1. Source of DNAs : lane 1 and 2, human (H.S.) male (M) and female (F) ; lane 3 and 4 ; male and female chimpanzees (Ch) ; lane 5 to 14, male and female lemurs. Less DNA was loaded in track 3 accounting for the weaker hybridization signal in female than in male chimpanzee.

female and one male DNA (lanes 8 and 9). In another female DNA however (lane 7) an additional 2.5 kb band was seen, while the dosage of the 4.4 kb band was reduced with respect to the DNAs in lanes 8 and 9. In a male DNA (lane 10) a similar pattern was present, however the supplementary fragment was different (3.25 kb). This suggests that the probe detects a restriction fragment length polymorphism with three allelic fragments (the 4.4, 3.2 and 2.5 kb TaqI fragments) and that both females and males can be heterozygous at this locus. Such an observation and the absence of dosage differences between males and females would be expected for an autosomally coded sequence and not for an X linked sequence.

DISCUSSION

The X and Y specific sequences which correspond to the DXS31 probe have interesting characteristics which can be examined at the light of cur-

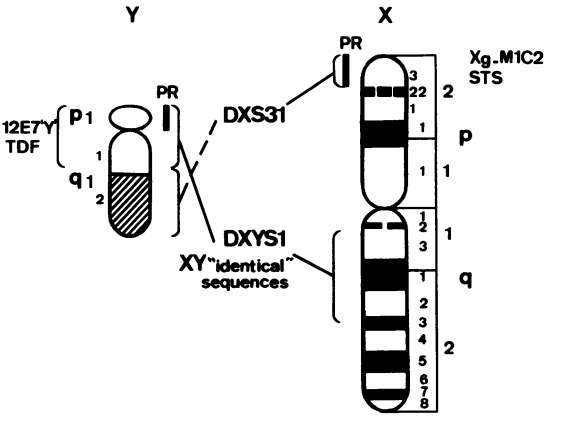


Fig. 6. Map of the human X and Y chromosomes. This diagram schematizes the localisation of the X and Y specific sequences corresponding to DXS31, DXYS1 (22) and to the XY "identical" sequences (20, 21). The broken line indicates partial sequence homology. The localisation of other relevant loci is also given : M1C2 and 12E7 "Y" are the X and Y genes controlling expression of the 12E7 antigen, STS : steroid sulfatase, Xg : blood group, TDF : testicular differenciation factor. The extent of the pairing region (PR, see ref 35) is indicated by the heavy vertical line.

rent hypothesises about homologies between the X and Y chromosomes (2, 3), and by comparison with other recently characterized X-Y homologous sequences (20-24). The cloned sequence is located on the X chromosome in the Xp22.3-pter region, the region which pairs with the Y at meiosis. However the Y specific fragments appear located in the heterochromatic region (q11-qter) and not in the pairing segment (see Fig. 6). Other probes (cloned from the Y chromosome) have recently been characterized which detect identical or almost identical sequences on the X chromosome (20), while the X and Y specific sequences corresponding to DXS31 have an estimated homology of 80 % only. Most or all of these X-Y "identical sequences" are located on the X chromosome in the q13-q22 region of the long arm (21) and at least four of them are also located in the euchromatic Ypter-q11 region (unpublished observations). The DXYS1 probe isolated by Page et al. (22) apparently belongs to the same class of sequences, with similar localisation in Xq and in the short arm of the Y chromosome (23). Thus the characteristics of these X-Y identical sequences is drastically different from those of the sequence we have isolated (see Fig. 6). An other X linked clone has however been very recently described (24) which shares some properties with DXS31.

It is located within the p21-pter region of the X chromosome (which includes p22.3-pter) and hybridizes to Y linked fragments which are of different sizes than the X specific ones. The Y sequences have been provisionally assigned to the heterochromatic part of Yq (quoted in ref 24). Thus although several cases of homologies between DNA fragments on the X and Y chromosomes have been described, no sequences have been found located on the pairing segment of both sex chromosomes (DXS31 being the first to be assigned precisely to the p223-pter region of the X chromosome). It is possible that the pairing observed at meiosis does not necessitate extensive sequence homologies, however the unexpected finding of such homologies between Xp22.3-pter and Yq11-qter on the one hand, and between Xq13-q22 and Yp on the other hand, is certainly intriguing.

The characteristics of the DXS31 probe are of particular interest for the study of anomalies of sex determination in man, especially in the cases of men with a 46 XX karyotype. It has been postulated for a long time that XX maleness could arise through translocation of Y material carrying a testis determining gene, onto the X chromosome (25, 26). This translocation could be the result of an accidental equal or unequal crossing over occuring within the pairing region of the two chromosomes (3). Evidence supporting such an X-Y interchange has been provided by the finding in some XX males of an anomalous segregation at the Xg locus and more recently by the demonstration that one XX male had inherited the Y-linked locus controlling the expression of the 12 E7 antigen (27). To test the generality of such a mecanism it is important to determine whether Y sequences are present in the genome of XX males and are carried by one of the X chromosomes, and also whether sequences have been deleted from the tip of the short arm of the X chromosome. DNA probes which detect Y specific sequences have proven extremely useful for the analysis of the sex reversed mutation in mouse (28) and it has been shown recently that the genome of 3 XX males out of 4 analysed contained various overlapping regions of the Y chromosome (17). The DNAs from the same patients were probed with DXS31 and were found to contain a double dose of the X linked fragments, and no Y linked fragment. It can be concluded that the Y linked sequence is located outside from the region of the Y chromosome which can be found in XX males, as could be expected from the assignment of the testis determining locus to the euchromatic part of the Y chromosome (pter-q11) (see Fig. 6). The X linked fragments are also outside a putative deleted region in the X chromosome (it should be noted that in one of the cases studied, the XX male did not inherit the paternal

Xg^a allele (17), which could suggest a deletion at this locus). However more cases should be studied since this could test the hypothesis that various translocation break points should be found in the distal region of the X chromosome in XX males, and could allow to order the various loci in this region (3).

Finally the preliminary analysis of sequences related to DXS31 in primates leads to interesting conclusions. The good conservation of hybridization signal between man and lemurs (which are thought to have separated in evolution for about 70. 10^6 years, see ref 29) suggests that the DXS31 sequences are functionally important and could correspond to an expressed gene : even with the lowest estimate of the rate of neutral evolution (4.10^{-9} per site per year), functionless unique DNA sequences should not show homology between the two species detectable under the assay conditions used (unless the overal rate of evolution has decreased in lemurs, see ref 29). Nucleotide sequence analysis of the X and Y linked fragments might tell whether one or both sequences can code for a protein. There is in fact some evidence that the heterochromatic region of the Y chromosome might contain gene(s) important in spermatogenesis (30).

The presence of the same male-female differential pattern in chimpanzee and man indicates that a sequence was present in the Y chromosome before divergence of the two species. In contrast the Y fragment detected by the DXYS1 probe in man which is almost identical to the fragment present in the long arm of the X chromosome is apparently not present in the Y chromosome of chimpanzee (quoted in ref 31). This could suggest that the almost perfect homology seen between sequences of the Xq13-q22 and Yp regions is due to a very recent chromosome rearrangement which occured after the divergence of chimpanzee and man, while a sequence related to DXS31 has been in the Y chromosome for a much longer period, as suggested also by the estimated 20 to 25 % divergence in sequence between the X and Y linked fragments. Finally in lemurs, the pattern seen with the DXS31 probe suggest autosomal linkage. This is somewhat unexpected in view of the great conservation of X linkage in mammalian species. Two possible explanations can be envisaged to this apparent breaking of the "law" first stated by Ohno (1). The X linked sequence is located in man in the p22.3-pter region where the genes which escape inactivation in females are located. These genes can function at the same dosage as autosomal genes and there would be no constraint against shifting of such a region between autosomes and the X chromosome. In fact the steroid sulfatase gene which is present in the same p22.3-pter region in

man might be autosomally coded in mouse and in marsupials, although this is the object of some controversy (31-34). Another possibility however would be that in lemurs the DXS31 related sequences are present identically on both X and Y chromosomes (except for rare polymorphisms such as found by TaqI digestion) either because they are present in a region where crossing overs between the X and Y are allowed or because of a recent gene conversion. It has been proposed that such a region of free (or even obligatory) exchange between the X and the Y chromosomes could be present in the distal part of the pairing regions, and would contain genes with a pseudo-autosomal pattern of inheritance (2). Direct evidence for the presence of such genes is lacking with the possible exception of the genes controlling the expression of the 12E7 antigen (6). Direct localisation of DXS31 related sequences in lemurs will tell whether they are autosomally coded or X-Y identical, and might lead to a better understanding of the evolution of sex chromosomes.

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