Volume 13 Number 15 1985 Nucleic Acids Research

Homologies between X and Y chromosomes detected by DNA probes: localisation and evolution

M.Koenig, J.P.Moisan, R.Heilig and J.L.Mandel

Laboratoire de Génétique Moléculaire des Eucaryotes du CNRS, Unité 184 de Biologie Moléculaire et de Génie Génétique de l'INSERM, Institut de Chimie Biologique, Faculté de Médecine, 11, rue Humann, 67085 Strasbourg Cédex, France

Received 21 May 1985; Revised and Accepted 16 July 1985

ABSTRACT

We have isolated and characterized DNA probes that detect homologies between the X and Y chromosomes. Clone St25 is derived from the q13-q22 region of the X chromosome and recognizes a 98% homologous sequence on the Y chromosome. Y specific fragments were present in DNAs from 5 Yq^- individuals and from 4 out of 7 XX males analysed. An X linked TaqI RFLP is detected with the St25 probe (33% heterozygosity) which should allow one to establish a linkage map including other polymorphic X-Y homologous sequences in this region and to compare it to a Y chromosome deletion map. Probe DXS31 located in Xp223-pter detects a 80% homologous sequence in the Y chromosome. The latter can be assigned to Yq11-qter outside the region which contains the Y specific satellite sequences. ACT1 and ACT2, the actin sequences present on the X and Y chromosomes respectively, have been cloned. No homology was detected between the X and Y derived fragments outside from the actin sequence. ACT2 and the Y specific sequence corresponding to DXS31 segregate together in a panel of Y chromosomes aberrations, and might be useful markers for the region important for spermatogenesis in Yq. Various primate species were analysed for the presence of sequences homologous to the three probes. Sequences detected by St25 and DXS31 are found only on the X chromosome in cercopithecoidae. The sequences which flank ACT2 detect in the same species autosomal fragments but no male specific fragments. It is suggested that the Y chromosome acquired genetic material from the X chromosome and from autosomes at various times during primate evolution.

INTRODUCTION

It is a widely held view that the human X and Y chromosomes have evolved from a common ancestor. Sequence homologies might thus exist between the sex chromosomes, as functionally important remnants of this evolutionary history. It has been suggested that such homologies should predominantly exist in Xp223-pter and Yp regions which pair at meiosis (1). The presence of genes encoded by the Y chromosome which are functionally related to genes located in Xp223-pter which specify the X_G blood group and the MIC2 surface antigen fit with this hypothesis (2). Several DNA fragments have been isolated from one sex chromosome which detect sequences with variable degree of homology on the other chromosome (3-8). Most of the latter homologies do

not reside in the pairing regions (3, 4, 5, 9, 10, 11). This raises the question of their origin and of their biological significance. Are they remnants of a common ancestral origin or the result of a more recent exchange of material between sex chromosomes ? Do they correspond to expressed sequences that do not need a dosage compensation or are they the reflect of high plasticity of non functional sequences through evolution.

We characterise here probes that detect DNA sequences on both the X and Y chromosomes with respect to their chromosomal sublocalisation and their evolution in different simian species. The data suggest that homologies in different regions on the sex chromosomes result of separate exchange events which occured at various times during primate evolution.

MATERIAL AND METHODS

1. Origin of cell lines and DNAs

DNA or blood samples from XX males, XX true Hermaphrodites, individuals with a Yq^- chromosome or a dicentric Yq chromosome were gifts of Drs. G. André (Strasbourg), J. Weissenbach (Institut Pasteur, Paris) and M.G. Mattéi (Marseille). The karyotype and sex phenotype of these patients are given in the results, table 3. DNA from AMIR 2N a human-mouse hybrid cell line (2) which contains the translocation Xqter-p22.3:Yq11-qter as the only human sex chromosome was received from Dr. P. Goodfellow (London).

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 was obtained from Dr. M. Fellous (Institut Pasteur, Paris).

DNAs from chimpanzees were a gift of Dr. J. Weissenbach. Blood samples of Macaca Mulatta (rhesus), M. Nemestrina (pigtail), M. Arctoïdes (stumptail), and M. Tonkeana were provided by Dr.Herrenschmitt (Strasbourg). Blood samples of Cercopithecus Vervett were a gift from Dr. Moulin (Institut Mérieux, Lyon). DNAs from two males and two females were studied for each of the cercopithecoids species, except for M. Arctoïdes of which only one male was available.

2. Preparation of DNA and Blot Hybridization.

Total genomic DNAs were extracted from cultured cell lines or human leukocytes, digested to completion with restriction endonucleases and fractionated by electrophoresis on 1.0% agarose gels, as previously described (12). Blotting onto diazobenzyloxymethyl paper (DBM) was performed according to Alwine et al. (13) without in situ depurination of the DNA. The DBM

papers were washed before hybridizations according to Bellard et al. (14). Hybridization buffer contained 40%-50% formamide (depending of the probe), 0.9 M sodium chloride, 50 mM sodium phosphate pH6.5, 2 mM EDTA, 0.08% (w/v) each of bovine serum albumin, Ficoll and polyvinylpyrrolidone, 4% dextran sulfate and 200 µg/ml of denatured salmon sperm DNA. Hybridization was performed at 42°C for 16-48 h, DBM papers were then washed at 60-68°C in the presence of 0.1 to 2 X SSC and 0.1% SDS, depending on the probes.

3. Origin and preparation of probes.

The origin of the probes is summarized in Table 1. The St25 clone contains a 6.1 kb EcoRI fragment derived from the X chromosome library of Davies et al. (17) and was isolated as not reacting with nick-translated total human DNA (18). A 1.25 kb and a 2.8 kb TaqI-MspI fragments were subcloned in the ClaI site of pBR322 (clone St25/1 and St25/2 respectively, see Fig. 1). Usually, inserts were separated from the vector sequences on sucrose gradient before being used as probes. The Y specific actin sequence is contained in a 5.2 kb EcoRI fragment. It was obtained from a genomic library of size selected 4 to 7 kb EcoRI fragments cloned in bacteriophage λ1149 and screened with a hamster cytoskeletal actin cDNA probe (19). The sequence was further identified as containing the 3.3 kb HindIII fragment

Table I : Origin and characteristics of probes used in this study

Human gene mapping symbol (15)	Probe name	Source	Insert size in kb.	Insertion site in pBR322	Stringency of washing °C/xSSC
-	St25/1	this study	1.25	ClaI	65°/0.1
-	St25/2		2.8	ClaI	65°/0.5
ACT2	Y Actin/1		1.05	-	65°/0.5
	Y Actin/2		1.3	-	65°/0.5
DXS31	M1A	Koenig et al. (3)	2.1	EcoRI-PstI	60°/1
DXS17	S21	Drayna et al. (16)	7.5	EcoRI	65°/0.5
DXYS1	pDP31	Page et al. (6)	4.5	"	65°/0.2
DXYS2	7b	Bishop et al. (7)	3	"	65°/0.5
DXYS7	13d		5	"	65°/0.2
DXYS8	115		1.6	"	65°/0.5
DXYS9	8j		5	"	65°/0.2

previously detected as Y chromosome specific (22), and was subcloned in pBR322 (J.P. Moisan, in preparation). The 1.05 kb SacI fragment (YAct/1) and the 1.3 kb HhaI-EcoRI fragment (YAct/2) which flank the actin sequence and which lack repetitive sequences were separated by polyacrylamide gel electrophoresis, recovered by electroelution and used as probes.

RESULTS

The St25 probe.

Detection of X and Y chromosome specific sequences

The 6.1 kb EcoRI fragment, St25, detects by hybridization to Southern blots of human DNAs under low stringency conditions a complex pattern of about 50 fragments. Under high stringency conditions (washing at 68°C in blots of human DNAs under low stringency conditions a complex pattern of about 50 fragments. Under high stringency conditions (washing at 68°C in 0.1 X SSC) a simpler pattern is obtained. Using the latter conditions, hybridization to DNA from normal males, normal females and from cell lines with 48 XXXX, 49 XXXXY and 49 XYYYY karyotypes showed that a 6.1 kb EcoRI fragment was present on the X chromosome and a 7 kb fragment was Y chromosome specific. A map of the St25 clone was constructed (Fig. 1) by hybridizing TaqI and MspI restriction fragments to Southern blots of human DNAs. This showed that the middle repetitive sequences correspond to an internal 1.8 kb region, while flanking sequences detected more specifically the X and Y linked fragments (Fig. 2). The two fragments St25/1 and St25/2 were subcloned in pBR322 and were used in further experiments.

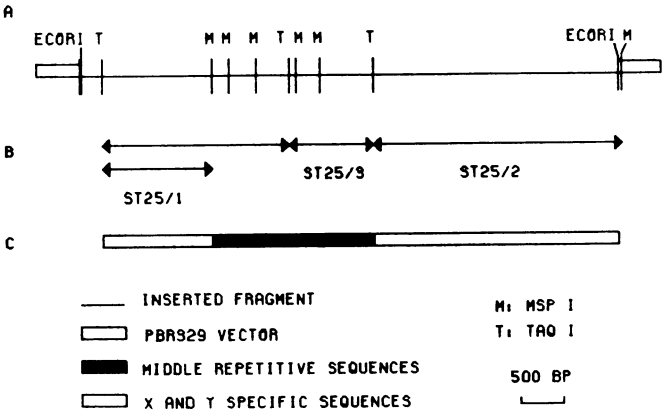


Fig. 1 : Map of the St25 clone. a) Restriction map. b) Subfragments used as probes (see Fig. 2). c) Localisation of middle repetitive sequences.

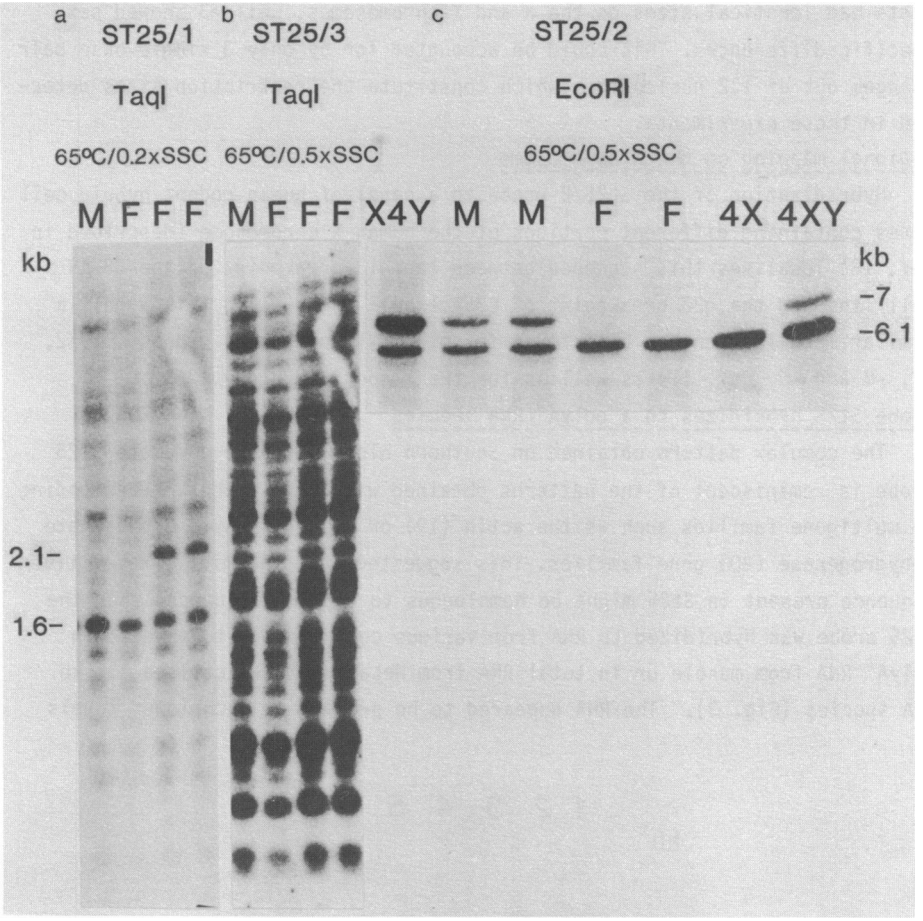


Fig. 2 : Hybridization pattern observed on Southern blots with 3 subfragments of the St25 probe (see Fig. 1). DNAs were prepared and analysed as described in material and methods. a and b. Subfragments St25/1 and /3 were successively hybridized to the same filter of TaqI digested DNAs and washed at the stringency indicated on top of the figure. M : Males, F : Females. c. Dosage analysis of EcoRI digested genomic DNA fragments hybridizing to St25/2. Source of DNA : M, unrelated normal males; F, unrelated normal females; X4Y, 4X, 4XY : lymphoblastoïd cell lines with 49 XYYYY, 48 XXXX and 49 XXXXY karyotype, respectively. All the detected fragments are shown.

When the sex chromosome specific probe St25/2 was hybridized to male and female DNAs digested with 9 restriction enzymes, we found no sex differences in PvuII, BglII, SacI, ScaI, StuI and XmnI digests. Only EcoRI, TaqI and Tth111I revealed one male specific fragment and one X linked fragment (as shown by difference in dosage in male and female DNA). In summary 9 frag-

ments had identical sizes on the X and Y chromosomes, while 3 showed sex specific differences. This could be accounted for by only 3 single base pair changes out of 122 nucleotides which constitute the restriction sites detected in these experiments.

Regional mapping on the X chromosome

Hybridization of the St25/2 probe to a panel of human-rodent hybrid cell lines containing different portions of the human X chromosome (described in ref. 18) localises this sequence between the q13 breakpoint of the 58-A9 cell line and the q22 breakpoint of C35A.1 cell line. Using this panel, a similar localisation has been found for other XY homologous probes (DXYS2, -7, -8 and -9, ref. 11) as well as for the X specific probe DXS17.

Probe St25 hybridizes to a polyA$^+$ RNA species

The complex pattern obtained on Southern blots with the complete St25 probe is reminiscent of the patterns obtained with cDNA probes corresponding to multigene families such as the actin (19) or glyceraldehyde 3 phosphate dehydrogenase (20) gene families. This suggested that the middle repetitive sequence present in St25 might be homologous to an mRNA sequence. When the St25 probe was hybridized to RNA from various cell types, it detected in polyA$^+$ RNA from muscle or in total RNA from HeLa and MCF-7 cells a 1.6 kb RNA species (Fig. 3). The RNA appeared to be present at much lower levels

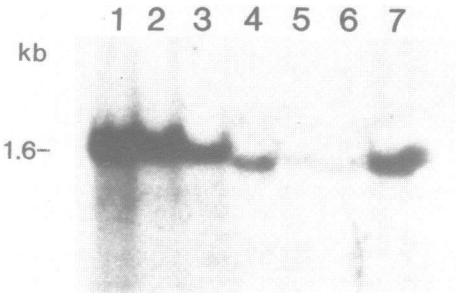


Fig. 3 : Detection of a RNA species homologous to St25 among RNAs of different cell types. RNA samples were electrophoresed on a 1.5% agarose gel containing 10 mM methylmercury hydroxide, blotted onto DBM paper and hybridized as described (21, 13) to St25 nick-translated probe. The paper was then washed in 0.5 X SSC and 0.1% SDS at 60°C. Source of RNA : lanes 1, 2 and 3 : poly A$^+$ RNA from human muscle (1 and 2) and horse muscle (3). Lanes 4, 5, 6 and 7 : total RNA from human cells. 4 : HeLa cells (15 µg), 5 : placenta (15 µg), 6 : fibroblasts (35 µg) and MCF7 (a cell line derived from a mammary carcinoma, 35 µg).

in human fibroblasts and placenta. Its sequence appears conserved in evolution since RNA of the same size was easily detected in horse skeletal muscle. Because of the large number of genomic fragments which hybridize to St25, we do not know whether this RNA is transcribed from X, Y or autosomal sequences.

Probes which flank the Y specific actin sequence.

We have previously shown that actin sequences were present on both the X chromosome (in the region p11-q11) and the Y chromosome (22). However, because of the complexity of this multigene family (about 50 actin sequences are detected in the human genome, many of which probably correspond to processed pseudogenes) it was not possible to know whether the presence of actin sequences on both sex chromosomes was a chance event or was due to the existence of another region of homology between the two chromosomes. We have recently cloned the X and Y linked genomic actin sequences (R. Heilig, J.P. Moisan, in preparation). From the 5.2 kb EcoRI fragment which contains the Y specific actin we could isolate two flanking sequences which lack repetitive elements and could thus be used as probes in genomic blotting experiments.

The two probes, Y Actin/1 and /2, detect only Y specific sequences as shown by hybridization to DNAs of the dosage panel described in Fig. 2C. Although probe /1 is entirely contained within a single TaqI fragment larger than 5.2 kb (the size of the original EcoRI cloned fragment), it reveals two Y specific fragments in male DNA digested with TaqI, which suggests a duplication of this particular sequence on the Y chromosome (Fig. 4). Under the same conditions, probe /2 detects a single TaqI fragment. Under washing conditions of high stringency, the 4.9 kb TaqI fragment detected by probe /1 disappears; therefore, it is likely to be less homologous to the probe than the 6.2 kb TaqI fragment.

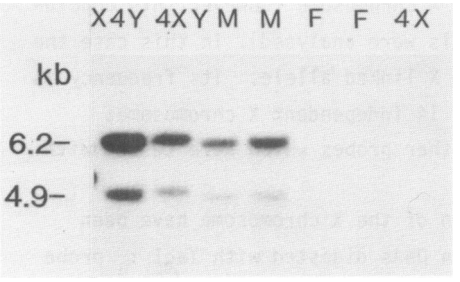


Fig. 4 : Dosage analysis of genomic DNA fragments hybridizing to Y Actin/1. DNAs were digested with TaqI enzyme. The origin and denomination of DNAs are the same as in Fig. 2C.

Towards a genetic map of the region in Xq13-q22 which shows high homology to the Y chromosome.

It would be of interest to establish a genetic map of the region on the X chromosome long arm which is homologous to part of the Y chromosome, since this would allow to compare the organization of the homologous sequences in the two sex chromosomes. As a preliminary step in this direction we have searched for restriction fragment length polymorphisms (RFLP) using the St25 probe and 4 other XY specific probes which have a similar localisation on the X chromosome long arm and show the same high degree of homology (~ 97%) between X and Y coded sequences (probes DXYS2, -7, -8 and -9, ref. 11).

In human DNA digested with TaqI, the St25/1 probe (which corresponds to a 2.1 kb TaqI fragment) detects a variable pattern in both females and males, determined by the presence or absence of 1.6 kb and 2.1 kb fragments. Females can have one or both of these fragments. On the contrary out of 84 males, 57 showed only the 1.6 kb fragment, 27 showed both the 1.6 kb and the 2.1 kb fragment, but none were found with only the 2.1 kb fragment. This suggested that a restriction fragment length polymorphism exists on the X chromosome with two allelic fragments and that on the Y chromosome, only the 1.6 kb fragment is found. This was confirmed both by segregation analysis in informative families and by the analysis of the frequency of the various fragments in male and female population. The frequency of the rarer allele on the X chromosome is found to be the same for males (11 out of 44 independent X chromosomes) and females (21 out of 93) if one assumes that there is no polymorphism on the Y chromosome. From the frequency of the X linked alleles in the caucasian population studied one can calculate that 33% of females should be heterozygous for this RFLP.

Similarly we detected a RFLP with probe DXYS2 (7b) in DNAs digested with PstI. This RFLP seems also limited to the X chromosome (However this conclusion is tentative since only 21 individuals were analysed). In this case the Y fragment has the same size as the rarer X linked allele. Its frequency is 21% in the small sample studied (3 out of 14 independent X chromosomes tested). No RFLPs were found with the 3 other probes which were tested with at least 11 restriction enzymes.

Two other probes in the q13-q22 region of the X chromosome have been previously shown to detect common RFLPs on DNAs digested with TaqI : probe DXYS1 is a XY homologous probe similar to St25 and DXYS2 (6), while probe DXS17 is X chromosome specific (16). We have thus at our disposal 4 polymor-

phic markers and it should be possible to establish their respective position by multipoint linkage analysis. Preliminary results indicate that recombination events can be found relatively easily between these markers. In particular two recombination events were detected between the X and Y homologous loci St25 and DXYS1 in 24 meioses analysed (Table 2).

Localisation on the Y chromosome.

In order to map the Y specific DNA fragments detected by the various probes, we have analysed their presence in various structural anomalies of the Y chromosome (summarized in Table 3) by Southern blotting experiments. In particular, we have studied XX males and true hermaphrodites since it has been recently shown that some of them contain Y specific sequences in their genome (23). The Y chromosome region present in such patients should contain the gene(s) controlling testicular differentiation (the TDF Locus). We have also analysed males which present a shorter long arm of the Y (Yq^-). Yq^- chromosomes are lacking the heterochromatic region which contains Y specific satellite DNA sequences. They can be found in normal fertile males but they are present at an increased frequency in sterile azoospermic males. It has been suggested that in the latter case an additional region containing gene(s) involved in spermatogenesis is deleted (24). However, cytogenetic analysis is often insufficient to differentiate between the two types of Y chromosomes. Finally, two translocations with breakpoints located in the long arm of the Y chromosome were also included in the localisation panel.

In addition to the three probes isolated in our laboratory (St25/2, Y Actin flanking sequences and DXS31) we have also used three previously described probes DXYS1 (6), DXYS7 and DXYS8 (7) that are XY homologous but show XY differential pattern with the TaqI enzyme. This allowed us to define 4 regions in the euchromatic part of the Y chromosome (Table 3). The first

Table II : Recombination analysis between loci in the Xq13-q22 region

pair of loci	number of meiosis	lod score	Θ max
St25/DXYS1	24	2.2	0.08
St25/DXS17	13	1.2	0.15
DXYS2/DXS17	10	1.3	0.10

Θ max is the recombination fraction for which the maximal lod score is obtained. The lod score is the log of the relative probability of linkage at Θ = Θ max.

TABLE III Analysis of Y chromosome deletions/translocations with probes detecting Y specific fragments.

karyotype	46XX								46XYq⁻					t(X;Y) (p22.3;q11)	46XY short Y	t(Y;15) (q11.1;p11)
sex phenotype	true hermaphrodites		sterile males												fertile male	normal female
identification	M169	GA12	M166	GA8 (11)	GRB027 (12)	GA1 (19)	GA7	M167	12	CHM004 (22)	SL (23)	LL92 (24)	17	38	AMIR2N	CHM007 (26)	CHM008 (27)
DXYS7	−	−	−	+	+	+	+	+	+	+	+	+	+	+	−	+	−
DXYS8	−	−	−	+	+	+	+	+	+	+	+	+	+	+	−	+	−
ST25	−	−	−	−	−	+	+	+	+	+	+	+	+	+	−	+	−
DXYS1	−	−	−	−	−	−	−	−	−	+	+	+	+	+	−	+	−
DXS31	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	−
Y ACTIN	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	−

The presence (+) or absence (−) of Y sequences homologous to the probes was tested as shown in Figure 5. Case 26 lacks and case 27 contains the Y satellite sequences DYZ1 and DYZ2 (25). The case number corresponding to ref. 25 is given in brackets.

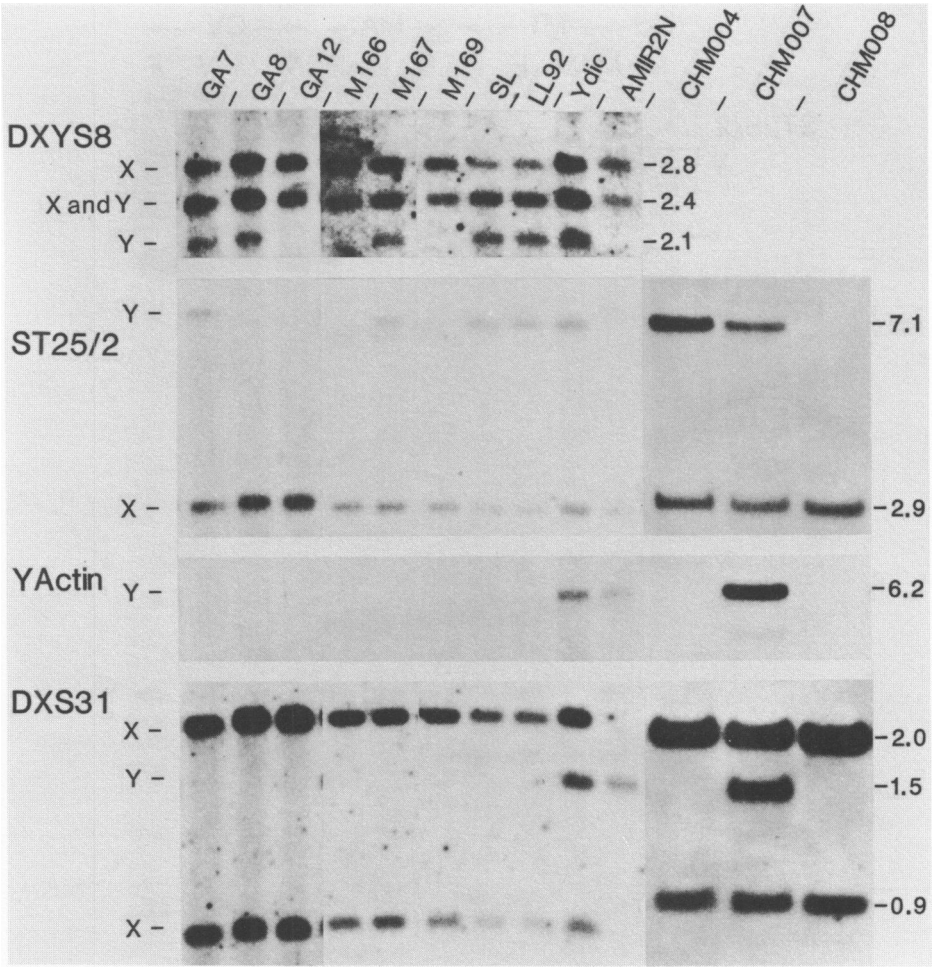
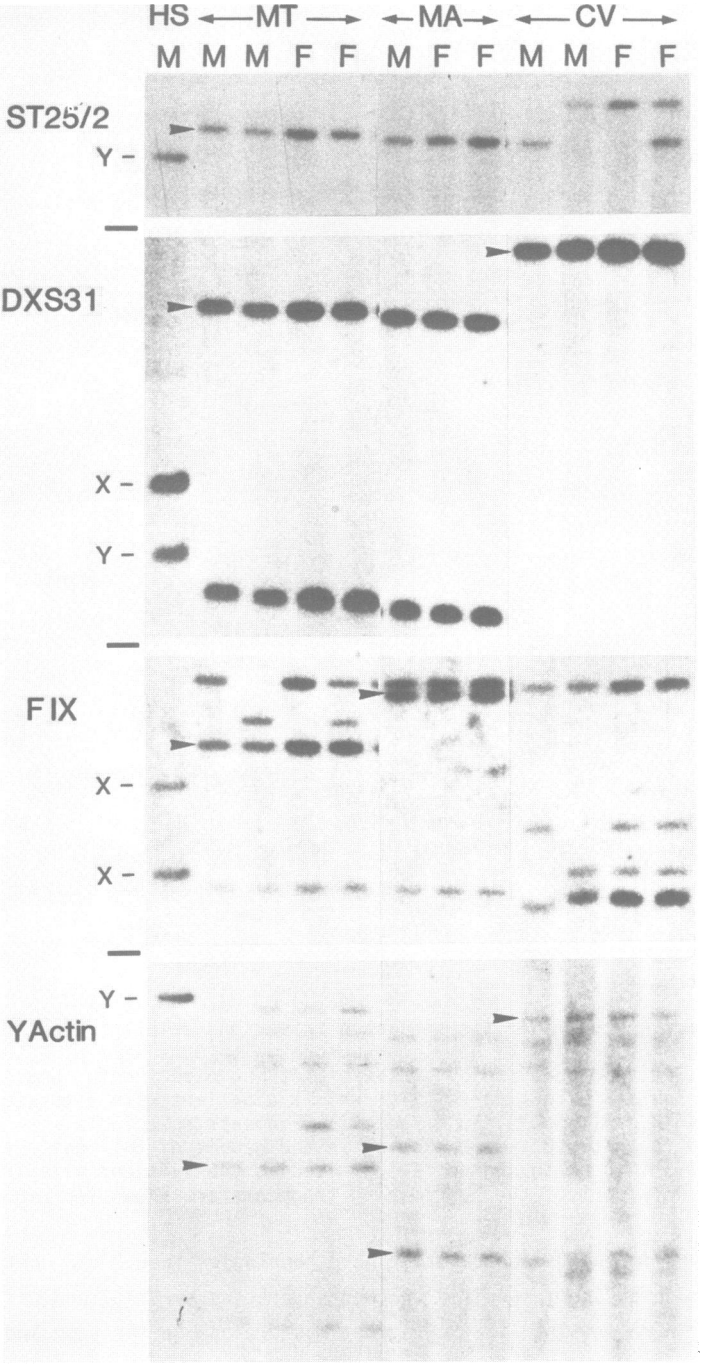


Fig. 5 : Search for Y specific sequences in various deletion/translocation of the Y chromosome with XY homologous probes. The DNAs were digested with the enzyme TaqI and analysed by Southern blotting experiment. Source of DNAs : see Table 3 except for "Y dic" who is a patient with a mosaic karyotype 45,X/46,X,dic(Y)(p11). No Y specific fragments detectable with the probes used in this study were missing in the genome of this patient. The chromosomal origin of the bands is indicated at the left and size (in kb) on the rigth. Only part of the hybridizing fragments are shown for probe DXS8.

three regions include sequences that have homologies in q13-q22 portion of the X chromosome. The Y specific fragments detected by DXYS7 and DXYS8 were found in 6 XX males. The St25-Y fragment was present in the same individuals with two exceptions (XX males GA8 and GRB027) (Fig. 5). In contrast, the Y


HS
MT
MA
CV
M M M F F M F F M M F F
ST25/2
Y -
DXS31
X -
Y -
FIX
X -
X -
Y -
YActin

specific fragment detected by DXYS1 was absent from all XX males, as found also in a similar study (25). All the above fragments are present in the Yq^- chromosomes.

On the other hand, the Y actin flanking sequences and the Y fragments detected by DXS31 were absent in all XX males and in the 5 sterile males with Yq^- chromosome, but were present in one fertile male with a Yq^- chromosome lacking the 3.4 kb and 2.1 kb repeat satellite sequences. In addition, they allow to differentiate between the breakpoints of the two translocations studied. These two probes are then localised in the euchromatic part of the long arm of the Y chromosome and might serve as markers for the region involved in spermatogenesis.

Search for homologous sequences in primates.

The analysis of the presence, in sex chromosomes of primates, of sequences related to those described above might shed some light on the origin of the XY homologous regions observed in man and distinguish between the alternative hypothesis of sequence conservation due to selective pressure or of recent exchange of genetic material between sex chromosomes.

We had previously reported that probe DXS31 detected X and Y specific sequences in chimpanzee as in man, but revealed sequences with an autosomal dosage in lemurs (3). We have now analysed DNAs from several cercopithecoïd species (macaca and cercopithecus). In all cases we saw no male specific bands (Fig. 6) and dosage demonstrated unequivocally that the sequences related to DXS31 are X linked (taking as a standard for densitometry scanning the fragments homologous to X linked coagulation factor IX gene, data not shown). Similarly, the St25/2 probe showed only X linkage in the cercopithecoïd species. In DNA from a male and a female chimpanzee a single EcoRI fragment was observed, of the same size as the human X linked fragment. However the data are insufficient to exclude the presence of a fragment of identical size on the Y chromosome of chimpanzee.

Actin flanking sequences showed a different evolutionnary pattern. Y

Fig. 6 : Conservation of sequences homologous to DXS31, St25/2 and Y Actin flanking sequences in cercopithecoïds. DNAs were digested with TaqI and analysed by Southern blotting. A cDNA probe for human coagulation factor IX gene (FIX) was used as a control for X linked sequences. Source of DNAs with respect to the sex : M, Males; F, Females; with respect to the species : HS, Homo sapiens; MT, Macaca tonkeana; MA, Macaca arctoïdes; CV, Cercopithecus vervett. X and Y refer to the chromosomal origin of the fragments detected in the human DNA. Arrows indicate the bands analysed by densitometric scanning (For DXS31 and FIX a lesser exposed autoradiogram was used in order to avoid saturation of the X Ray film). All hybridizing fragments are shown for primate DNAs, except for the FIX probe.

Actin/2 probe detects a simple male specific band in chimpanzee of the same size as the fragment seen in man under the same hybridization conditions. However, in cercopithecoïds a complex pattern of mostly or only autosomal bands was revealed with no male specific fragments (Fig. 6). The differences observed between individuals from the same species are not sex specific but are probably due to restriction site polymorphisms.

DISCUSSION

We have studied three probes which detect regions homologous between the X and Y chromosomes. The St25 fragment derived from the q13-q22 region of the X chromosome is highly homologous (98%) to sequences of the Y chromosome, probably in Yp and contains also a middle repetitive sequence homologous to a polyA$^+$ 1.6 kb long RNA species. The DXS31 originating from the Xp22.3-pter region shows only 80% homology to sequences present in Yq11. Finally, we show that the presence of actin sequences on both X and Y chromosomes is not related to an extended homology between these two chromosomes since sequences which flank the Y actin sequence are completely male specific in man. The St25 probe provides a marker for study of the region close to the testicular differentiation factor which is present in many (or all) XX males, while DXS31 and the Actin related Y specific sequences provide useful markers for the region of the long arm involved in spermatogenesis.

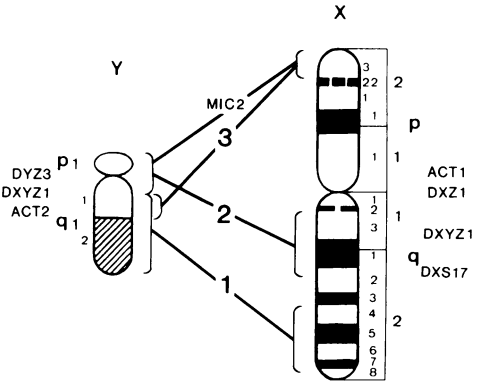


Fig. 7 : Scheme of the regions of homology between the X and Y human chromosomes. Region 1 corresponds to clone Puc9HI (5). Region 2 contains clones DXYS1 to -9 (9, 11), St25, cosmid 75 (10). Region 3 contains DXS31 (3) and DXS69 (4). ACT1 and ACT2 correspond to the X and Y linked actin sequences. DXZ1 and DYZ3 are alphoïd repeats (15). DXYZ1 is described in (15). MIC2 is the antigen recognized by the 12E7 monoclonal antibody (27).

From our results and those previously reported we can draw a tentative scheme of the localisation and evolution of various homology regions found in sex chromosomes. Cooke et al. have isolated DNA fragments which appear identical in both X and Y chromosomes (no base change in 1 kilobase pair) and which are located in Xq24-qter and in Yq (region 1 on figure 7) (5). An important collection of probes, which include DXYS1 (Page et al., 6), DXYS2 to -9 (Geldwerth et al., 7), cosmid 75 (Wolfe et al., 8) and the probe St25 described here share very similar properties : (i) localisation in q13-q22 region of the X chromosome (q13-qter for cosmid 75) and in the euchromatic pter-q11 region of the Y chromosome, (ii) very high but not absolute homology between the X and Y linked sequences (estimates of homology are in the 97-99% range). The number of clones originating from this region (numbered 2 on figure 7), isolated from a Y chromosome specific library (Bishop et al., 26) suggests that it may constitute an important part (up to one quarter) of Y euchromatin. Some but not all of the probes from this region detect Y specific sequences in some but not all XX males. For instance in this study we showed that Y sequences corresponding to probe St25 are present in 4 XX males out of 7 while Y specific fragments corresponding to probe DXYS7 and DXYS8 are present in 6 of these subjects. On the other hand, DXYS1 detects no Y specific sequences in any XX males so far tested (J. Weissenbach, D. Page, personal communication and table 3). This should allow to construct a deletion map of this region in the Y chromosome. Since, as described here and by Page (6), several of these probes detect restriction fragment length polymorphism on the X chromosome, it should be also possible to construct a linkage map of the X chromosome region and to compare the order of the various sequences in sex chromosome. This should shed some light on the organisation of the homologous regions and their origin. Finally, two probes, DXS31 (3) and DXS69 (probe 71-7A, ref. 4) map in Xp22.3-pter and detect partially homologous sequences in Yq (region 3). We show here that Y linked sequences homologous to DXS31, located in the q11-qter region, are proximal to the heterochromatic region which contains the Y specific satellite sequences, DYZ1 and DYZ2.

In order to distinguish between various hypothesis concerning the origin of these homology regions we have analysed the sequences corresponding to St25 and DXS31 in several primate species. In fact, both probes detect in cercopithecoïds only X linked sequences. In chimpanzee, it has been shown that probe DXS31 detects both X and Y specific sequences (3). Although we do not know at present the status of sequences corresponding to St25 in

chimpanzee, D. Page has shown for analogous DXYS1 locus that it is only X linked in this primate species (9). Thus it may be suggested that region 2 arose through a transposition event from the X chromosome to the Y chromosome which occured after the divergence of chimpanzee and man. On the other hand, region 3 is the result of a more ancient event which occurred after the divergence of cercopithecoïds and hominoïds 30-35 million years ago, in agreement with the 80% homology found between X and Y linked sequences. Our finding that the Y actin flanking sequences are homologous to autosomal sequences in cercopithecoïds indicate that exchange of sequences were not limited to the sex chromosomes.

In conclusion, it appears as if a substantial part of the Y chromosome is made of rather recently acquired sequences. The Y chromosome might in fact encode very few specific genes. However, as suggested by P. Burgoyne (personal communication) very small Y chromosomes might lead to an increase in the frequency of non disjonction events at meiosis and have been therefore selected against.

ACKNOWLEDGMENTS

We thank Drs. J. Weissenbach and M. Fellous (Institut Pasteur, Paris), Dr. G. André (Strasbourg), Dr. M.G. Mattei (Marseille), Dr. P. Goodfellow (ICRF, London) and Dr. Moulin (Institut Mérieux, Lyon) for generous gifts of blood samples, cell lines and DNAs and Drs. K. Davies and R. Williamson for allowing us to use the X chromosome library. We also wish to thank Dr. Herrenschmitt (Unité de primatologie de l'Université Louis Pasteur, Strasbourg) for his generous gift of blood of Macaques.

We are grateful to C. Sengel and C. Kloepfer for excellent assistance and to C. Aron and B. Boulay for help in preparing the manuscript. This work was supported by grants from the Fondation pour la Recherche Médicale Française.

REFERENCES

1. Burgoyne, P.S. (1982) Hum. Genet. 61, 85-90.
2. Goodfellow, P., Banting, G., Sheer, D., Ropers, H.H., Caine, A., Ferguson-Smith, M.A., Povey, S. and Voss, R. (1983) Nature 302, 346-349.
3. Koenig, M., Camerino, G., Heilig, R. and Mandel J.L. (1984) Nucleic Acids Res. 12, 4097-4109.
4. Kunkel, L.M., Tantravahi, U., Kurnit, D.M., Eisenhard, M., Bruns, G.P. and Latt, S.A. (1983) Nucleic Acids Res. 11, 7961-7979.
5. Cooke, H.J., Brown, W.A.R. and Rappold, G.A. (1984) Nature 311, 259-261.
6. Page, D., De Martinville, B., Barker, D., Wyman, A., White, R., Francke, U. and Botstein, D. (1982) Proc. Natl. Acad. Sci. USA 79, 5352-5356.
7. Bishop, C., Guellaen, G., Geldwerth, D., Fellous, M. and Weissenbach, J. (1984) J. Mol. Biol. 173, 403-417.
8. Wolfe, J., Erickson, R.P., Rigby, P.W.J. and Goodfellow, P.N. (1984) The EMBO J. 3, 1997-2003.

9. Page, D.C., Harper M.E., Love, J. and Botstein, D. (1984) Nature 311, 119-123.
10. Wolfe, J., Erickson, R.P., Rigby, P.W.J. and Goodfellow, P.N. (1984) Ann. Hum. Genet. 48, 253-259.
11. Geldwerth, D., Bishop, C., Guellaen, G., Koenig, M., Vergnaud, G., Mandel, J.L. and Weissenbach, J. (1985) The EMBO J., in press.
12. Grunebaum, L., Cazenave, J.P., Camerino, G., Kloepfer, C., Mandel, J.L., Tolstoshev, P., Jaye, M., De la Salle, H. and Lecocq, J.P. (1984) J. Clin. Invest. 73, 1491-1495.
13. Alwine, J.C., Kemp, D.J., Parker, B.A., Reiser, J., Renart, J., Stark, G.R. and Wahl, G.M. (1979) in Methods in Enzymology, Wu, R. Ed., Vol. 68, pp. 220-242, Academic Press.
14. Bellard, M., Kuo, M.T., Dretzen, G. and Chambon, P. (1980) Nucleic Acids Res. 8, 2737-2750.
15. Human Gene Mapping, Los Angeles Conference (1984) Cytogenetics and Cell Genetics 37.
16. Drayna, D., Davies, K., Hartley, D., Mandel, J.L., Camerino, G., Williamson, R. and White, R. (1984) Proc. Natl. Acad. Sci. USA 81, 2836-2839.
17. Davies, K.E., Young, B.D., Elles, R.G., Hill, M.E. and Williamson, R. (1981) Nature 293, 374-376.
18. Oberlé, I., Camerino, G., Kloepfer, C., Moisan, J.P., Grzeschick, K.H., Hellkuhl, B., Hors-Cayla, M.C., Van Cong, N., Weil, D. and Mandel, J.L. (1985) Hum. Genet. (in press).
19. Hanauer, A., Levin, M., Heilig, R., Daegelen, D., Kahn, A. and Mandel, J.L. (1983) Nucleic Acids Res. 11, 3503-3516.
20. Hanauer, A. and Mandel, J.L. (1984) The EMBO J. 3, 2627-2633.
21. Bailey, J.M. and Davidson, N. (1976) Anal. Biochem. 70, 75-85.
22. Heilig, R., Hanauer, A., Grzeschick, K.H., Hors-Cayla, M.C. and Mandel, J.L. (1984) The EMBO J. 3, 1803-1807.
23. Guellaen, G., Casanova, M., Bishop, C., Geldwerth, D., Andre, G., Fellous, M. and Weissenbach, J. (1984) Nature 307, 172-173.
24. Tiepolo, L. and Zuffardi, O. (1976) Hum. Genet. 34, 119-124.
25. Vergnaud, G., Page, D., Simmler, M.C., Brown, L., Rouyer, F., Noel, B., Botstein, D., de la Chapelle, A. and Weissenbach, J. (1985) Am. J. Hum. Genet. (in press).
26. Bishop, C.E., Guellaen, G., Geldwerth, D., Voss, R., Fellous, M. and Weissenbach, J. (1983) Nature 303, 831-832.
27. Human Gene Mapping 6, Oslo Conference (1982) Cytogenet. Cell Genet. 32.