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Evolution of homologous sequences on the human X and Y chromosomes, outside of the meiotic pairing segment

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ABSTRACT

A sequence isolated from the long arm of the human Y chromosome detects a highly homologous locus on the X. This homology extends over at least 50kb of DNA and is postulated to be the result of a transposition event between the X and Y chromosomes during recent human evolution, since homologous sequences are shown to be present on the X chromosome alone in the chimpanzee and gorilla.

INTRODUCTION

The mammalian X and Y (sex) chromosomes are structurally and genetically distinct from each other, but are thought to have evolved from an homologous pair (1). Genetic investigation of the 12E7 antigen (2) and observations on the pairing behaviour of the human X and Y chromosomes at male meiosis (3) suggest that homology will be maintained between them in the pairing segment (Xpter and Ypter). In meiosis a single obligate crossover was postulated between the X and Y in this region, possibly as a requirement for correct segregation of the chromosomes (4). This prediction has been borne out by the isolation of such X-Y homologous DNA sequences from the pairing region of the human sex chromosomes, which do indeed show a "pseudoautosomal" pattern of inheritance consistent with their exchange at meiosis (5).

Molecular analysis of the human Y chromosome has revealed however that X-Y homology is not restricted to the meiotic pairing segment (6,7,8). Indeed a majority of sequences isolated at random from the human Y chromosome share some degree of homology with the X and are located on parts of the chromosome thought not to be involved in meiotic recombination. The mechanistic basis for such homology must therefore differ from that involving the "pseudoautosomal" sequences.

Cooke et al (9) described the isolation of a sequence (2:13) from the human Y chromosome which shares a high level of homology with the X. To date we have found no detectable differences between the X and Y copies of this

sequence, which are located at Xq26-q27 and Yqcen-q11.1. Here we show that this homology extends for at least 50kb and an evolutionary comparison of 2:13 related sequences in the genomes of higher primates shows them to be located on the X chromosome alone in these species.

These observations lead us to suggest that 2:13 sequences were involved in a transposition event from the X onto the Y chromosome which occurred in recent human evolution after the divergence of the human lineage from those of the chimpanzee and gorilla. This may have been part of a large-scale event which brought about the appearance of several other X chromosome sequences onto the Y (10,11).

MATERIALS AND METHODS

Origins of mammalian cells and DNAs

3E7 (12) and 853 (13) are somatic cell hybrids carrying the human Y chromosome as the only cytologically detectable human chromosome, on mouse and Chinese hamster backgrounds respectively. Similarly ThyB.1-33-C1 12 is a mouse x human hybrid in which the human X chromosome is retained by selection with hypoxanthine aminopterin and thymidine (14).

Male chimpanzee and male and female gorilla primary fibroblasts were a gift from J. Delhanty (London).

697 x 175, 750 and 367 DNAs (somatic cell hybrids carrying defined segments of the human X chromosome) were a gift from H. Ropers (15). 445 x 393 and isoYP DNAs were a gift of P. Goodfellow.

Southern blotting

DNAs were digested with restriction enzymes according to the manufacturers specifications, electrophoresed on agarose gels and transferred to nitrocellulose (Schleicher and Schull) or Hybond N (Amersham).

Hybridisations were carried out in 0.1M Tris-HCl pH7.8 0.75M NaCl, 5mM EDTA, 0.1% Ficoll, 0.1% polyvinylpyrrolidine, 0.1% BSA, 0.1% SDS, 0.1% sodium pyrophosphate and 10% dextran sulphate at 68°C. Probes were radiolabelled with ^{32}P by nick translation (16) or random priming (17).

Library construction

Insert DNA was size selected on sucrose gradients prior to cloning, and libraries constructed and screened using standard procedures (18). pJB8 vector was prepared by the method of Ish-Horovicz and Burke (19).

RESULTS

Human chromosomal localisation of 2:13 sequence

Cooke et al (20) described the construction of a library from flow-sorted human Y chromosomes in λgtwes and the isolation from this library of a

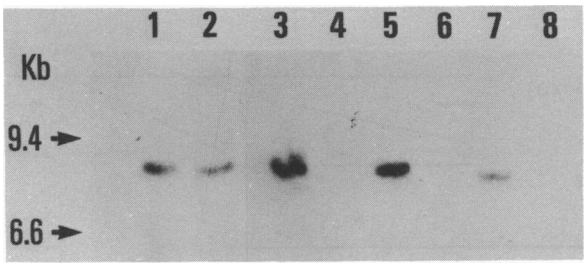

Figure 1. Chromosomal localisation of 2:13
Genomic DNAs were digested with EcoRI, electorphoresed and transferred to Hybond N (Amersham). The filters were hybridised to ^{32}P-labelled pG15 and washed at 0.1 x SSC, 68°C. Lane 1, human ♀ ; 2, human ♂; 3, ThyB1; 4, mouse parent of ThyB1; 5, 3E7; 6, revertant of 3E7 lacking the human Y chromosome; 7, 853; 8, revertant of 853 lacking the human Y chromosome.

recombinant phage λY2:13. A single copy probe, pG15, was obtained by subcloning a Sau3A1 partial digest of λY2:13 into pUC9. pG15 detects a single 8.3kb EcoRI fragment in Southern hybridisation to male and female human DNAs. The Y chromosome localisation of 2:13 is confirmed by hybridisation of pG15 to DNA from somatic cell hybrids which carry human Y chromosomes. Hybridisation to female DNA thus indicates that 2:13 related sequences must also be present on an autosome or the X chromosome. pG15 hybridises to DNA from a somatic cell hybrid carrying the X chromosome as its only cytologically detectable human component confirming the latter possibility (Fig. 1).

TABLE 1

REGIONAL CHROMOSOME LOCALISATION 2:13

Cell line		Hybridisation to pG15
697 x 175	Xqter - p21	✓
750	Xqter - q26	✓
367	Xqter - q28	0
iso Yp	X + Yp	>0.4 copies/diploid cell
445 x 393	Xqter-p22.3 + Yq11.1-qter	>0.5 copies/diploid cell

NOTE: Copy numbers were ascertained by densitometry relative to the signal from total human and ThyB1 DNAs

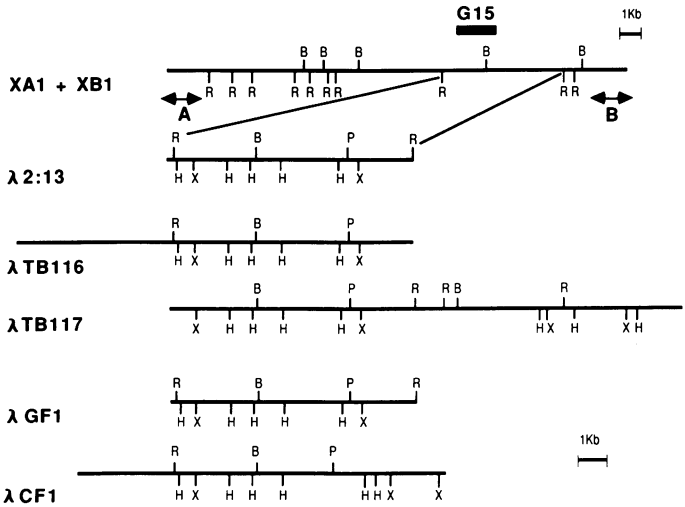

Figure 2. Restriction maps of λ2:13 and related clones

Restriction maps of the inserts of clones described in the text are shown. λY2:13 is derived from the human Y chromosome, ; λTB116 and 117 and cosmids XA1 and XB1 are from the human X and λCF1 and λGF1 are from chimpanzee and gorilla DNAs respectively. The relative position of pG15 is also shown.

R = EcoRI; B = BamHI; H = HindIII; X = Xbal; P = PstI

Identification of sequences held in common between the sex chromosomes, is not in itself surprising: X-Y meiotic pairing is thought to be directed by sequence homologies between Xpter and Ypter with recombination between these regions being a prequisite for disjunction (4). In situ hybridisation data (9) and Southern hybridisation of pG15 to somatic cell hybrids retaining different segments of the human X and Y chromosomes (Table 1) localises 2:13 homologous sequences to Xq26-q27 and Yqcen-11.1. This is well outside of any region thought to be involved in meiotic recombination (3).

Level of human X-Y homology defined by λY2:13

To establish the degree of homology between the X and Y located copies of 2:13, the X sequence hybridising to pG15 was isolated from an EMBL4 library of ThyB.1-33 C1 12 (a cell hybrid carrying the human X chromosome on a mouse background) DNA. Two clones (λTB116 and 117) hybridising with pG15 were isolated. These clones, together with λY2:13, were restriction mapped by digestion with multiple restriction enzymes and by partial digest mapping with synthetic oligonucleotides to the left and right hand of cos sites of λ

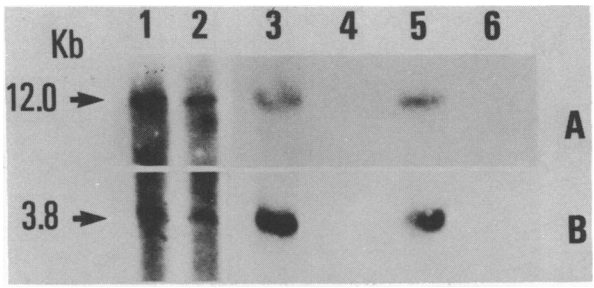

Figure 3. Extent of X-Y homology surrounding the 2:13 locus.
Southern hybridisation of fragments A and B from clone XB1 (Fig. 2) to DNAs from 1, human ♀; 2, human ♂; 3, ThyB1; 4, mouse parent of Thybl; 5, 3E7; 6, revertant of 3E7 lacking the human Y chromosome.
An EcoRI digest of XB1 was run on a low gelling temperature agarose gel and fragments A and B excised. These gel slices were radio-labelled by random priming (17) then preannealed to human DNA to remove signal from repeated sequences (26) prior to hybridisation.

(21,22). At this level of analysis, no differences were detectable between the X and Y homologous loci (Fig. 2).

After subcloning into suitable vectors, parts of λTB116 and λY2:13 were sequenced by Maxam and Gilbert, and Sanger dideoxy sequencing techniques (23,24). No base changes were observed between the X and Y derived clones in 1200bp of sequence from corresponding regions of each clone. This puts a lower limit of 99.92% on the level of homology between the human X and Y chromosomes at the 2:13 loci. Such a high degree of homology might be expected if these loci were functional such that selection pressure were operating to conserve their sequences. If this were the case homologous sequences would probably be found in the mouse genome (25). Fig. 1 indicates that this is not so and pG15 does not detect any transcripts in poly A^+ RNA from a variety of human tissues (data not shown).

It is unlikely that regular recombination between the human X and Y chromosomes outside of the pseudoautosomal region is responsible for maintaining the homology between the two copies of 2:13. Due to the different structural and genetic makeup of the two chromosomes such an event would destroy the structural integrity of both chromosomes. Therefore the X-Y homology identified here may be the result of the relatively recent appearance of 2:13 related sequences upon both of the human sex chromosomes. In order to investigate this possibility we have further characterised sequences flanking the original probes in human and higher primate DNAs.

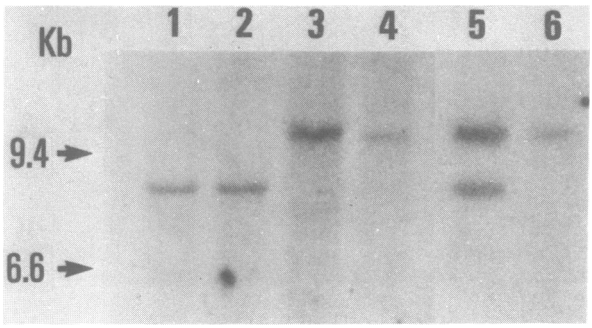

Figure 4. 2:13 related sequences in higher primates.
EcoRI digest of 1, human ♀; 2, human ♂; 3, chimpanzee ♀; 4, chimpanzee ♂; 5, gorilla ♀; 6, gorilla ♂ DNAs were hybridised to pG15. The dosage of hybridising DNA in each genome was quantitated by densitometry (Table 2).

The extent of human X-Y homology at the 2:13 loci

To analyse the extent of homology between the long arms of the human X and Y chromosomes at the loci homologous to 2:13, a Sau3A1 partial digest library of ThyB1 DNA was constructed in the cosmid vector pJB8 and two clones (XA1 and XB1) hybridising to pG15 isolated. The combined restriction maps of these clones, which overlap each other by >80%, are shown in Fig. 2.

A and B were hybridised, after stripping of repeated sequences (26), to Southern blots of human male and female DNAs and somatic cell hybrids carrying the human X and Y chromosomes (Fig. 3). By this criterion, human X-Y homology extends for at least 47kb around the 2:13 loci.

Nature of 2:13 sequences in higher primates

If the high level of X-Y homology at the 2:13 loci is due to the recent appearance of this sequence upon both of the human sex chromosomes, estimates for the molecular clock in higher primates, c0.2%/MY, (27) indicate that such an event must have occurred within the last one million years. This is well after the divergence of the human lineage from its closest relatives, the chimpanzee and gorilla.

Fig. 4 shows the hybridisation of pG15 to digests of male and female human, chimpanzee and gorilla DNAs. In order to determine the dosage of pG15 in each species, the signal in each track was quantitated by comparison of the signal from an X-specific probe λRC8 (28) and a Y-specific probe (p21A1, gift of B. Smith) on the same gel. Table 2 shows that 2:13 related sequences exhibit sex-linked dosage in the chimpanzee and gorilla and are therefore presumably located on the X chromosome alone in these species. The simplest

TABLE 2

Dosage of G15 sequences in male and female human, chimpanzee and gorilla DNAs

DNA	Ratios of DNA loadings on gels	Ratios of Peak intensities			Ratios of peak intensities corrected for loadings			copy number/ diploid cell		
		G15	21A1	λ RC8	G15	21A1	λ RC8	G15	21A1	λ RC8
Human ♀	1.00	1.00	-	1.00	1.00	-	1.00	2.0	-	2.0
Human ♂	0.80	0.97	1.00	0.44	1.21	1.0	0.55	2.4	1.0	1.1
Chimpanzee ♀	1.58	1.42	-	1.69	0.90	-	1.07	1.8	-	2.1
Chimpanzee ♂	0.99	0.43	1.02	0.52	0.43	0.82	0.53	0.9	0.8	1.1
Gorilla ♀	0.90	0.52 + 0.50	-	0.91	1.11	-	1.01	2.2	-	2.0
Gorilla ♂	1.00	0.47	0.88	0.48	0.49	0.85	0.48	1.0	0.9	1.0

explanation for these observations is that 2:13 sequences were transposed from the X onto the Y chromosome during recent human evolution.

Southern blots of pG15 show a 10kb EcoRI fragment in chimpanzee DNA in contrast to the 8.3kb fragment seen in human DNAs, whereas gorilla DNAs tested contain both of these fragments. The gorilla 8.3kb and chimpanzee 10.5kb fragments were isolated from appropriate phage libraries and the clones (termed λGF1 and λCF1 respectively) were restriction mapped (Fig. 2). Unfortunately the gorilla 10.5kb fragment proved refractory to our attempts to clone it. Part of λCF1 was sequenced and showed 2% divergence from the human 2:13 homologous clones (data not shown). This is the average divergence expected between human and chimpanzee sequences (27) and further argues against the existence of any strong selection pressure operating to prevent divergence of the X and Y located human sequences.

Branching orders for the chimpanzee/human/gorilla lineages are not universally accepted but the extensive work of Sibley and Ahlquist (27) suggests that gorillas may have diverged from a common human/chimpanzee ancestor c10MY ago, with chimpanzee/human branching occurring c5MY ago. therefore in a common ancestor to all 3 species, 2:13 sequences would have been present on the X chromosome alone. Of 4 gorilla X chromosomes examined in this study, 3 carry 2:13 sequences upon a 10.5kb EcoRI fragment the other chromosome carrying the 8.3kb form. Thus when the gorilla lineage split from

the common human/chimp ancestor both forms of 2:13 were carried on the X chromosomes contributing to present day gorillas. Of the 3 chimpanzee X chromosomes examined all carry the 10.5kb EcoRI fragment hybridising to pG15 but the existence of the 8.3kb form in the chimpanzee population cannot be ruled out. After the divergence of the human and chimpanzee lineages 2:13 sequences were transposed from the X to the Y chromosome, in the former lineage. All human X and Y chromosomes examined in this study (42 in total and probably all of Caucasian origin) carry 2:13 sequences upon an 8.3kb EcoRI fragment. Therefore unless such a transposition event occurred independently several times during human evolution, all human Y chromosomes we have examined must have originated from the single chromosome onto which the 2:13 sequence was transposed, since the Y chromosome is monosomic and inherited only from father to son.

DISCUSSION

We have described here the identification of a transposition event between the human X and Y chromosomes which has occurred so recently during human evolution that >99.9% homology still remains between the X and Y located copies of this sequence. This does not appear to have been an isolated event. Study of sequences isolated at random from the human Y chromosome has revealed against all expectations, that X-Y homologous sequences (excluding those from the pseudoautosomal region) represent a large proportion of the molecular content of the chromosome (6,7,8). Such sequences can be classified into 3 categories according to their chromosomal sublocalisation, level and extent of homology and chromosomal position in primates.

Category I sequences, which include 2:13, are defined here as those sequences detecting extensive, high levels of homology between the human sex chromosomes. All have been localised to Xq12-28 and the euchromatic part of the Y (Refs. 6,7,10,11,29,30,31,32), with a majority being found on Yp: 2:13 is therefore unusual in this respect since we have local ised it to Yqcen-q11.1. For only one category I sequence, DXYS1(10), aside from 2:13, it is known that the probe has an X only localisation in higher primates, although DXYS12 (11) is known to be on the X chromosome alone in the macaque. It can only be speculated as to whether, due to their similar characteristics, category I sequences were translocated onto the Y together in a single event.

The second category of X-Y homologous sequences e.g. DXS31 (33), DXS69 (34), GMGXY3 (7), DXYS18 and 18 (35) are those which detect homology between

the sex chromosomes at only low stringency. They are generally localised to Xp21-pter and the euchromatic part of Yq. In the one case examined, DXS31 (33), X-Y homology is also detected in the chimpanzee, but an X only localisation is found in the macaque. These sequences may therefore have been involved in an X to Y transposition event predating that involving category I sequences occurring prior to the divergence of the human and chimpanzee lineages but after the split of the higher primates from the old world monkeys. Category II sequences do not detect long contiguous stretches of X-Y homology (35) but this does not rule out their initially being part of a single transposition event. The long arm of the human Y chromosome has probably undergone extensive rearrangements accompanying the amplification of the DYZ1 and 2 repeats, since the divergence of the human and chimpanzee lines (36).

The last category of X-Y homologous loci and the last to be defined at a molecular level are those which were predicted to exist on the basis of the meiotic pairing behaviour of the X and Y chromosomes and on genetic evidence (2), and have been termed pseudoautosomal, since they do not show sex-linked inheritance (4). These sequences: DXYS14 (37), DXYS15 and 17 (38) and MIC2X/Y (39) have been shown to be located at the tips of the short arms of the sex chromosomes and to be regularly exchanged between these chromosomes at meiosis, with a gradient of recombination from the telomere down towards the centromere (5). This recombination maintains X-Y homology in this region of the sex bivalents and is probably the only remaining relic of the ancestral homology of the sex chromosome pair.

The human Y chromosome therefore appears to be a recent evolutionary patchwork with a previously unexpected large proportion of the chromosome sharing various degrees of homology with the X chromosome. Deletion maps constructed of the Y chromosome from aberrant chromosomes (40,41) have shown that Y-specific and X-Y homologous sequences are interspersed on the chromosome. Much of the effort directed towards the human Y chromosome has been to try to identify sequences involved in primary sex determination. It might be supposed that such a gene(s) will be specific to the Y chromosome alone with no homologous sequence on the X chromosome. The large proportion of clones isolated at random from the human Y chromosome which are homologous to the X are therefore unhelpful in this search. We are currently investigating methods of selectively cloning only Y-specific material from the Y chromosome, eliminating from our cloning strategy X-Y homologous sequences using the deletion enrichment procedure developed by Kunkel et al (42) (manuscript in preparation).

The molecular map of the human Y chromosome is probably the most complete of any human chromosome despite its lack of defined genetic loci. The use of XX males and XY females as resources for the generation of deletion maps of the chromosome will no doubt lead to the isolation of DNA sequences involved in directing the differentiation of indifferent embryonic gonads into testes. Such a gene would be a fine model for the study of developmental processes in mammals.

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