

# Differential expression pattern of XqPAR-linked genes *SYBL1* and *IL9R* correlates with the structure and evolution of the region

Maurizio D'Esposito<sup>1,+</sup>, Maria Rosaria Matarazzo<sup>1,+</sup>, Alfredo Ciccodicola<sup>1</sup>, Maria Strazzullo<sup>1</sup>, Richard Mazzarella<sup>2</sup>, Nandita A. Quaderi<sup>3</sup>, Hiroyuki Fujiwara<sup>4</sup>, Minoru S. H. Ko<sup>4</sup>, Lucy B. Rowe<sup>5</sup>, Angela Ricco<sup>6</sup>, Nicoletta Archidiacono<sup>6</sup>, Mariano Rocchi<sup>6</sup>, David Schlessinger<sup>2</sup> and Michele D'Urso<sup>1,\*</sup>

<sup>1</sup>International Institute of Genetics and Biophysics, CNR, 80125 Naples, Italy, <sup>2</sup>Department of Molecular Microbiology, Washington University Medical School, St. Louis, MO 63110, USA, <sup>3</sup>Telethon Institute of Genetics and Medicine, DIBIT, Via Olgettina 58, 20132 Milan, Italy, <sup>4</sup>Center for Molecular Medicine and Genetics, Wayne State University School of Medicine, 5047 Gullen Mall, Detroit, MI 48202, USA, <sup>5</sup>The Jackson Laboratory, Bar Harbor, ME 04609, USA and <sup>6</sup>Istituto di Genetica, Università di Bari, Via Amendola 165/A 70126 Bari, Italy

Received June 2, 1997; Revised and Accepted July 25, 1997

The recently discovered second pseudoautosomal region (XqPAR) contains at least two genes, *IL9R* and *SYBL1*. Recent findings show that, like XpPAR genes, *IL9R* escapes X inactivation and its Y allele is also expressed, but *SYBL1* seems to act like an X-linked gene, expressed from the active X chromosome but not from the inactive X or Y. Here we show that differences are also seen in the evolution of the sex chromosome locations of *IL9R* and *SYBL1*. *IL9R* is known to be autosomal in mice, and is X-linked only in primates. *SYBL1*, however, has been found to be on the X chromosome in all mammals tested, from marsupials to humans. Both genes were duplicated on the Y homologue of the terminal portion of the X chromosome during the evolution of *Homo sapiens* from other higher primates. The inactivation pattern of *SYBL1* may be correlated with its longer history of X linkage, and at a more centromeric chromosomal position during evolution; the more recent X linkage and more telomeric position of the *IL9R* gene may explain its autosomal, 'uninactivated' transcriptional status.

## INTRODUCTION

Sex chromosomes are thought to derive from a homomorphic pair of sex chromosomes, with gradual reduction of the Y chromosome in a complex multistep process recently called the 'addition-attrition hypothesis' (1). Homologous regions on both the long and short arms of the X and Y chromosomes attest to their common origin (2,3). Various lines of evidence (4,5) reveal that a special class of homology occurs in a region, the XpPAR, 2.6

Mb in length, that recombines between X and Y, ensuring correct segregation at male meiosis (6).

In the pseudoautosomal region (PAR), the requirement for dosage compensation, which underlies Ohno's law (7) of the conservation of genes on the X chromosome, is relaxed. As a result, addition-attrition could lead to variation in PARs among different species; in fact, the mouse homologues of two genes in the human XpPAR, *CSFR2A* and *IL3RA* (8,9) have been mapped to autosomes.

A second, 320 kb PAR at the Xq end of the chromosome recently has been characterized (10–12). Its evolutionary history is only partially known. Anonymous probes like *DXYS61* (3) showed X linkage in higher primates and presence on both human X and Y; but the inclusion of those probes in a PAR was only clear when DNA from the whole region was cloned (13). The sequence at the PAR boundary on the X and Y chromosomes indicates that the XqPAR may have arisen by transposition via illegitimate recombination between LINE sequences (12).

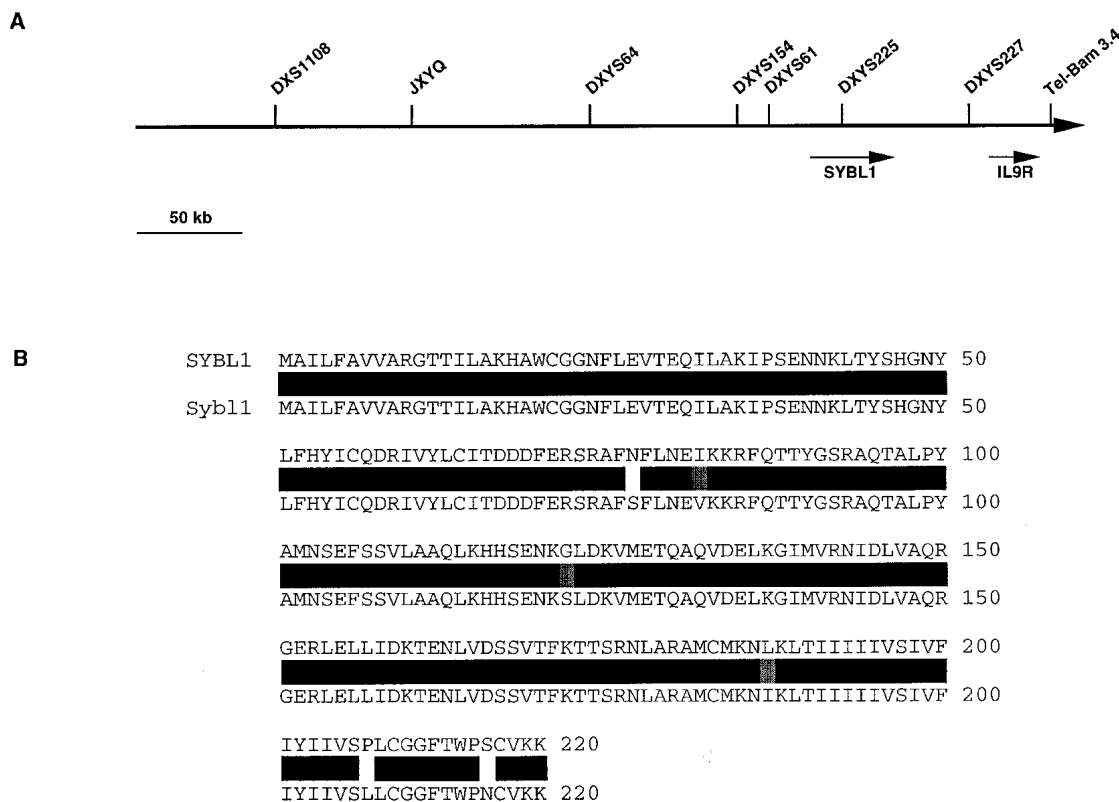
A more detailed analysis of the molecular history of the XqPAR has become possible with the isolation of two genes encoded in the region (14,15). In spite of their close linkage in the XqPAR, their transcriptional status is quite different: like XpPAR genes, *IL9R* escapes X inactivation and its Y allele is also expressed. In contrast, *SYBL1* seems to act like an X-linked gene; it is expressed on the active X chromosome but not from the inactive X or the Y.

*IL9R* was shown recently (16) to be autosomal in the mouse while it is X-linked in apes and X/Y-linked only in the human lineage. It thus represents a fourth case of a gene eluding conservation of X linkage in eutherian mammals.

In contrast, we show that, using the XqPAR synaptobrevin-like gene *SYBL1* (15) as an evolutionary marker, at least one XqPAR-linked gene shows conservation of X linkage among eutherian species. This coincides with previous observations in marsupials and monotremes for other Xq genes (17). In order to

\*To whom correspondence should be addressed. Tel/Fax: +39 81 7257247; Email: durso@iigbna.iigb.na.cnr

+These authors contributed equally to this work



**Figure 1.** (A) Schematic representation of the human XqPAR region. From left to right: *DXS1108* (11), *JXYQ* [XqPAR boundary marker (12)], *DXYS64* (30), *DXYS154* (11), *DXYS61* (3), *DXYS225* (22), *DXYS227* (22) and *TelBam.3.4* (31). (B) Protein alignment between human *SYBL1* and mouse *Syb11* genes. Solid bars indicate regions of identity and shaded bars indicate regions of conservative change.

explore the evolutionary history of the locus, we identified *SYBL1* homologues in marsupials, mice and higher primates, and we show that this gene is X-linked in each species studied. A further step in *Homo sapiens* progenitors added the corresponding segment to the Y to create the modern human XqPAR.

A bipartite structure and evolution of this region can thus be suggested that correlates with the striking differences in transcriptional behaviour of the two genes so far studied.

## RESULTS

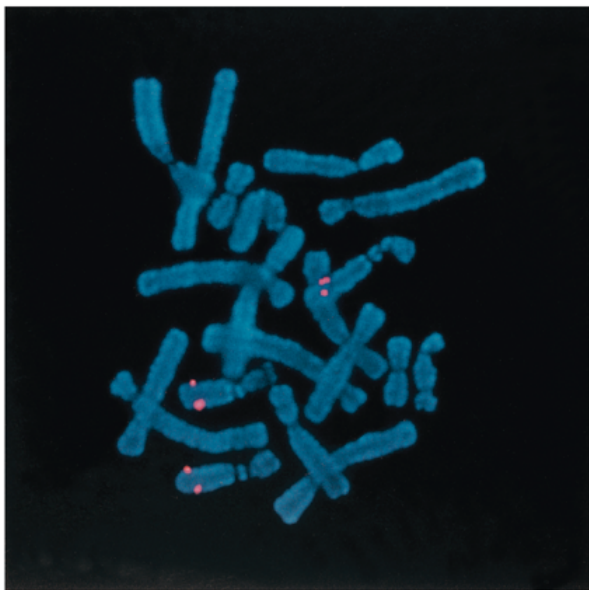
### Conservation of the synaptobrevin-like gene

Previous reports localized two genes, in the human XqPAR, *IL9R* and *SYBL1* (14,15), the former in a more telomeric position with respect to the latter (Fig. 1A). Using the human *SYBL1* cDNA sequence as a query, the BLAST (18) algorithm indicated a high degree of conservation for the *SYBL1* gene, with strong similarity to a plant synaptobrevin and weaker similarity to a cohort of other eukaryotic synaptobrevins (15), a class of proteins that provides specificity in targeting pathways within cells, including those of the nervous system. In experiments using the human cDNA as a probe, Southern blot hybridization with DNAs from a number of species showed comparably strong signals from *Arabidopsis*, chicken, rat, mouse, primates and humans, and fainter bands from yeast and *Drosophila* (not shown). Figure 1B shows the high level of conservation between the human and the newly isolated (Materials and Methods) mouse homologue. Both species have a

220 amino acid open reading frame (ORF) with identical start and stop codons. The 1583 bp mouse sequence is 82.1% identical at the nucleotide level with its human counterpart; and base substitutions are clustered mainly in the 5' and 3' untranslated regions (UTRs). A comparison of the mouse and human *SYBL1* putative proteins reveals six amino acid substitutions between the two species, three of which are conservative. This comparison defines the region of the probable transmembrane segment as comprising amino acids 189–206, since the human protein contains a proline at amino acid 207 that would disrupt the  $\alpha$ -helix beyond that point. Since members of the synaptobrevin family are thought to insert post-translationally into the endoplasmic reticulum (ER) via their C-terminus and are then transported to their site of function, this topology places amino acids 1–188 in the cytoplasm and amino acids 207–220 in the lumen of the ER. The absolute conservation of four cytoplasmic cysteines and two luminal cysteines raises the possibility that they are involved in intramolecular and intermolecular disulphide bonding, respectively.

### Localization of the synaptobrevin-like gene in marsupials

For most genes, the X chromosome of all eutherian mammals follows the predictions of Ohno's law (7) regarding the evolutionary conservation of genes on the X. Ohno suggested that the complement of genes on the X chromosome tends to stabilize over evolutionary time to avoid disrupting dosage compensation



**Figure 2.** FISH analysis on PtK1 female rat-kangaroo (*Potorous tridactylis apicalis*) kidney cell line using the cosmid c8.2 as a probe for *SYBLI*. The presence of three X chromosomes is most likely attributable to the use of an established cell line that has undergone some duplication; the conclusion of X linkage is in any case unaffected.

and sex determination. This suggestion has also been supported in marsupials and monotremes for a subset of eutherian X-linked genes, those located on the long arm of the human X (17). The isolation of the XqPAR gene, *SYBLI* (15), permitted us to extend the examination of this prediction, and to assess some features of the functional conservation of the X chromosome.

Using the human cosmid c8.2, which contains part of the human *SYBLI* gene (12,15), fluorescence *in situ* hybridization (FISH) on metaphase spreads from a kidney cell line derived from an adult female rat-kangaroo (*Potorous tridactylis apicalis*) showed signals at the distal end of the Xq (Fig. 2); note that the X chromosome is similar in length and centromere position to some autosomes, but is characterized by a large secondary constriction near the centromere in the long arm (19).

#### Genetic mapping of *SyblI* loci in the mouse genome

Primers designed from the 3'UTR of the murine *SyblI* cDNA were used to amplify DNAs from two related mouse species by PCR. *Mus spretus* genomic DNA produced a single amplification product of the expected size (see Materials and Methods). However, amplification of C57BL/6J genomic DNA produced a tight doublet of bands, the smaller of which co-migrated with the *M. spretus* amplification product. The unique C57BL/6J band was mapped in both reciprocal backcrosses from The Jackson Laboratory. Analysis of the haplotype data revealed that this *SyblI* locus mapped in the most proximal position on the mouse X chromosome (Fig. 3A). The combined BSS and BSB data (only hemizygous males could be scored from the BSB) show 2/137 recombination events between *SyblI* and *DXMit26/DXBir1*, placing *SyblI*  $1.46\text{cM} \pm 1.02\text{cM}$  proximal to *DXMit26/DXBir1* (Fig. 3B).

The second locus suggested by the doublet from the PCR of C57BL/6J DNA was mapped with a 0.5 kb probe from the 3'UTR of the murine *SyblI* cDNA hybridized to Southern blots of the same backcross DNAs. In both crosses, a strongly hybridizing polymorphic band (10.5 kb in *M. spretus* DNA versus 4.1 kb in C57BL/6 DNA) mapped to distal chromosome 2, co-segregating with *Gnas* (Fig. 3A). This chromosome 2 locus has been named *SyblI-ps*. Additional non-polymorphic *SyblI* bands seen in this analysis appear to correspond to the X-linked *SyblI* locus. The similarity of hybridization intensity suggests that both loci are highly similar to the probe.

To clarify further the nature of the two *SYBLI*-related loci in the mouse genome, a genomic phage library was screened with the entire mouse cDNA as a probe. Two of seven independent phage isolates were characterized by PCR, using primers specific for the cDNA positions 267–732, spanning exons 3–7, confirming the presence of two loci, one of them without introns, thus representing a probable pseudogene. FISH analysis of mouse chromosomes from the WP-G5194 cell line using these phages showed a specific unique signal close to the centromere of the X chromosome for the *SyblI* gene-specific phage,  $\lambda 5.1$ , hybridization with the putative pseudogene phage  $\lambda 2.1$  revealed a strong signal on chromosome 2 and a fainter signal on the X (data not shown). These data strongly suggest that the X-linked locus represents the original intact gene in the mouse genome.

#### Molecular analysis of *SYBLI* localization in primates

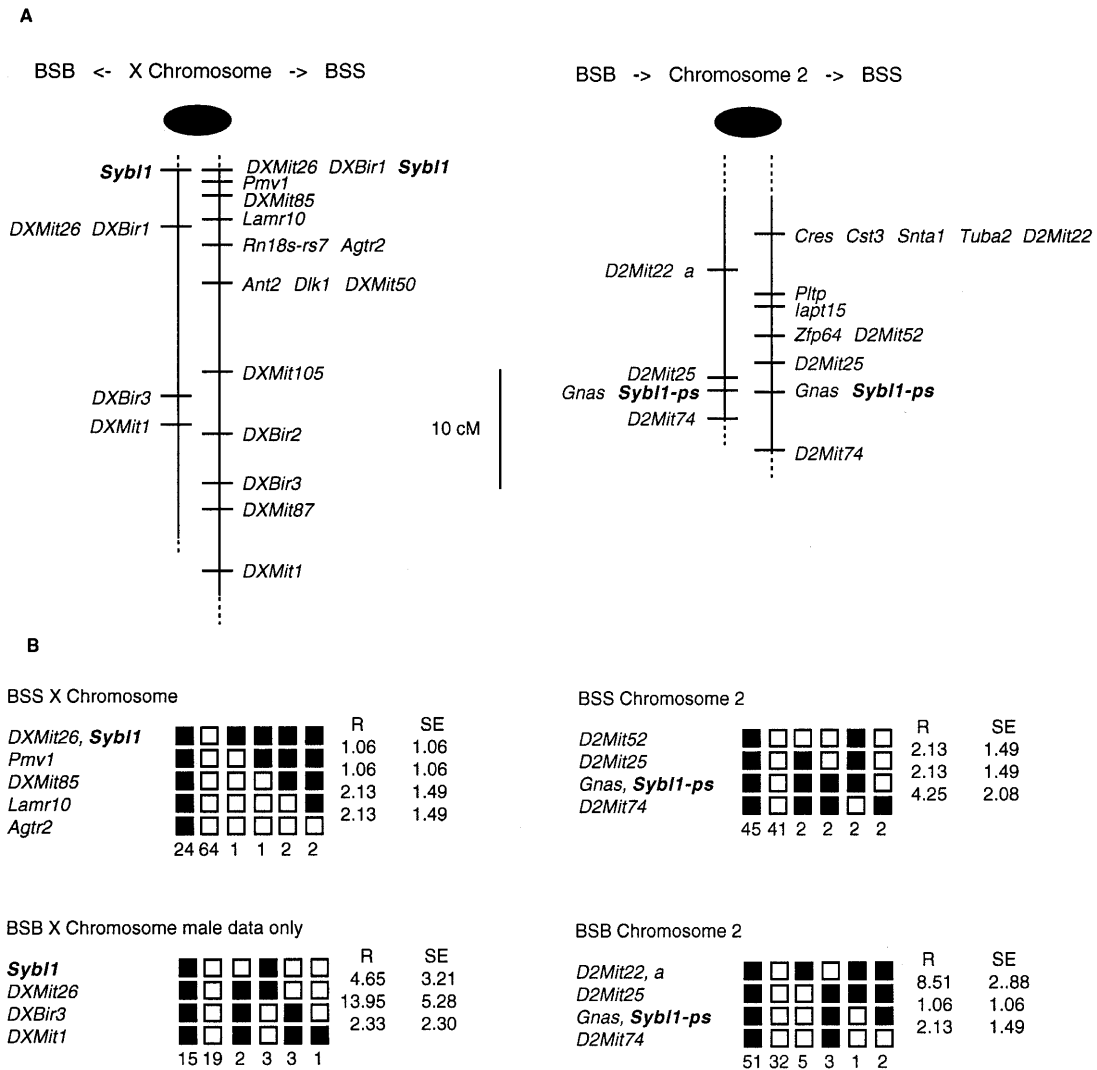
A probe derived from the cosmid c8.2 was hybridized *in situ*, as already described (20), to metaphase spreads of lymphoblastoid cell lines derived from chimpanzee (*Pan troglodytes*, PTR), gibbon (*Hylobates lar*, HLA), gorilla (*Gorilla gorilla*, GGO) and macaque (*Macaque fascicularis*, MFA). Signals are seen only in the Xq28 region in all cases (Fig. 4B). (Note that the signal on the GGO X is subtelomeric, displaced by a distal heterochromatic region in Xq28.)

No evidence of Y linkage was seen in any of these non-human species, suggesting that it was transferred to the Y after the divergence of the human lineage from the other apes. FISH analysis with the same cosmid on metaphase spreads from normal human male cells shows a signal at Xq28 and at the telomeric region of Yq (Fig. 4A). These results imply that the prospective XqPAR region was confined to the X chromosome until it was also added to the Y chromosome very recently in human evolution.

#### DISCUSSION

Recent studies on monotreme and marsupial chromosomes reveal that genes mapped on the long arm of the human X chromosome retain this linkage in these distantly related genomes (17). Those studies postulated the presence of an XCR (X conserved region) including all of Xq, beginning before or at the time of divergence between Prototheria, Metatheria and Eutheria, or ~170 million years ago (17). This can be taken as a confirmation and refinement of Ohno's law, though exceptions have also been noted (8,9).

Previous calculations (3,12), based on sequence divergence of the LINE element at the human XqPAR boundary compared with homologues, indicate that the chromosome translocation and subsequent X/Y inheritance for sequence elements in this region



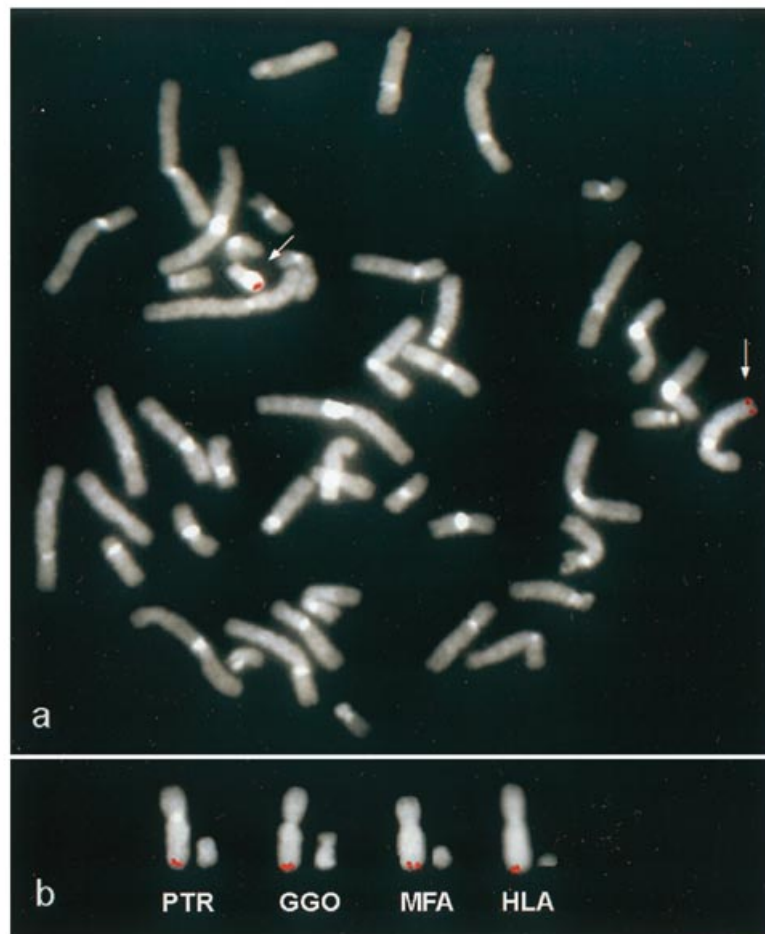
**Figure 3.** (A) Map figures from the Jackson backcrosses (27). The proximal end of the X chromosome maps and the distal end of the chromosome 2 maps are shown with the map from the BSB [(C57BL/6J×*Mus spretus*) F1×C57BL/6J] data on the left and from the BSS [(C57BL/6J×SPRET/Ei) F1×SPRET/Ei] data on the right in each figure. The maps are depicted with the centromere toward the top. A 10 cM scale bar is shown between the maps. Loci mapping to the same position are listed in arbitrary order. Missing typings were inferred from surrounding data where assignment was unambiguous. The BSB X chromosome map is based on data from male animals only ( $n = 43$ , see text). Raw data for linked loci are from the Jackson Laboratory Backcross Panel World Wide Web address <http://www.jax.org/resources/documents/cmdata>. (B) Haplotype tables from the Jackson backcrosses showing data for loci closely linked to *Syb1* on the X chromosome and loci closely linked to *Syb1-ps* on chromosome 2. Loci are listed in order, with the most proximal at the top. The black boxes represent the C57BL/6J allele and the white boxes the *M. spretus* allele. The number of animals with each haplotype is given at the bottom of each column of boxes. The percentage recombination (R, equivalent to cM) between adjacent loci is given to the right of the figure, with the standard error (SE) for each R. Missing typings were inferred from surrounding data where assignment was unambiguous.

occurred 15–20 million years ago. On the other hand, divergences between human, chimpanzee and gorilla lineages seem to have occurred more recently in evolution. The details are uncertain because expert opinion varies on the ‘star-like’ branching and timing of the process, but one estimate suggests a critical period 6–8 million years ago (21).

Earlier evidence for X–Y homologous DNA near the Xq/Yq termini in humans was found by Bickmore and Cooke (3), who showed that an anonymous probe *DXYS61* (formerly called 2:13) in the region was X-linked in great apes but was found on both X and Y in humans. Because the *DXYS61* sequence is limited to higher primates and is not expressed, this analysis was necessarily

limited, and only hinted at the possibility of more extensive homologies.

The use of the human *SYBL1* gene as a probe has permitted us to detect the mouse and marsupial homologues and determine some features of the molecular and functional evolution of the XqPAR. Apparently the conservation of syntenic equivalence in Xq extends to the human *SYBL1* gene, but not to the somewhat more telomeric gene, *IL9R* [16; the genes are ~ 40 kb apart (A.Ciccodicola *et al.*, in preparation)]. The simplest evolutionary pathway for the human XqPAR would start from a time at which the *SYBL1* gene was already at a subtelomeric position on the X. An *IL9R* gene copy would then have been interpolated from its



**Figure 4.** DAPI-banded human metaphase (a) hybridized with biotinylated c8.2 probe, revealing signals (in red) at the tips of Xq and Yq. In (b) are reported partial karyotypes (X and Y chromosomes) from common chimpanzee (*Pan troglodytes*, PTR), gorilla (*Gorilla gorilla*, GGO), macaque (*Macaca fascicularis*, MFA) and gibbon (*Hylobates lar*, HLA). Only signals at the tip of Xq were found.

earlier autosomal location (mouse chromosome 11 or its evolutionary equivalent) between the *SYBL1* locus and the telomere, before the divergence of human from the great apes (16). Then the region was duplicated on a Y chromosome homologue as well, possibly involving an illegitimate LINE-mediated recombination between X and Y chromosomes (12). Other additions in the XqPAR included the introduction of the proterminal repeat TelBam3.4 (16).

A PAR is defined by two criteria: a copy of the sequences is found on both the X and the Y chromosomes, and the region shows meiotic pairing and recombination between the X and the Y. Genetic exchange between the X and Y homologues of the XqPAR has been demonstrated by the use of polymorphic markers in the region (11,22). Ongoing sequencing efforts have revealed that three markers (see Fig. 1A) that have been used to detect polymorphism and recombination are respectively within the *SYBL1* gene (LH1, GDB name: DXYS225, between exons 5 and 6), closely linked to *SYBL1* (sDF1, GDB name: DXYS154), and very near to *IL9R* (LH2, GDB name: DXYS227). In addition, sequencing results show that the X and Y copies of the coding sequences of *SYBL1* are identical at the nucleotide level, consistent with the active homogenization of the content of this

part of the X and Y via recombination (A.Ciccodicola *et al.*, in preparation). In their currently extant forms, the XpPAR and XqPAR both show relatively high recombination rates though, unlike the XpPAR (5), the XqPAR does not show an obligate cross-over in every meiosis.

Our more detailed understanding from the mapping data of the physical evolution of the XqPAR can be used to address questions about the function of genes in this region. The most striking difference between the two genes so far studied in the XqPAR remains their modes of regulation of expression, that are reported elsewhere (15,16). *IL9R* expression seems to be typically pseudoautosomal, occurring from both X and Y copies; this may reflect its more recent introduction from an autosome to the X chromosome at this distal position. A sequence barrier may protect some X-linked genes, including genes in the XpPAR (23), from the passage of a wave of inactivation. If so, such a barrier could exist between the *SYBL1* and *IL9R* loci, since the X-linked copy of *SYBL1* is subject to X-inactivation and *IL9R* is not. The exact nature of such barriers is not yet known, but might include epigenetic changes, such as methylation of CpG islands, replication timing or absence of acetylated histone H4 (24–26), or other structural changes in chromatin.

In addition to the X inactivation, the unusual inactivation of the *SYBL1* copy on the Y chromosome suggests the presence of other mechanisms, that differ from those that inactivate X chromosome genes, since there is no known inactivation centre on the Y. The effect is to render the function of the *SYBL1* gene typical of normal X linkage, and to make the Y homologue a reservoir of alleles that can be recruited back to active form by recombinational exchange.

In conclusion, our analysis suggests that the XqPAR includes a highly conserved, older, more centromeric part of the X which has been extended by punctate evolution in the more distal portion. We have demonstrated that the XqPAR is a region newly added to the human Y and not a remnant of an ancient X–Y homology region, and we postulate that differences in expression of *SYBL1* and *IL9R* can be explained by differences in the time of transfer of these segments to the X and Y chromosomes, and the proximity of each to the telomere. The mechanisms of regulation of these genes remain to be analysed.

## MATERIALS AND METHODS

### Library screening

A 17 days embryonic mouse (Swiss Webster/NIH pooled embryos) cDNA library in  $\lambda$ gt10 (Clontech) was screened with a coding portion of the cDNA for the human *SYBL1* gene (15). Three recombinant phages were isolated, and only the largest clone, 1.6 kb in length was subcloned and sequenced completely.

### Sequence analysis

Mouse *Sybl1* cDNA was subcloned into pGEM-4Z vector (Promega Biotech) and analysed by cycle sequencing on an Applied Biosystems 373A automated sequencer. The cDNA sequence has been submitted to EMBL, accession No. X96737.

### Mouse backcross analysis

**PCR mapping.** The primers *sybl1F* (5'-TCCCATTGCAGTTGAT-TTGA-3') and *sybl2R* (5'-ATAGCTCATAAGACTAGCGG-CG-3') from the 3'UTR of the murine *Sybl1* cDNA were used to PCR amplify SPRET/Ei and C57BL/6J parental and the BSS and BSB backcross progeny genomic DNAs (27). Twenty five ng of template DNA was used in 35 cycles of 94°C, 20 s; 57°C, 40 s; and 72°C, 40 s, and the amplification products were then resolved through 3% SeaPlaque agarose gels (FMC).

**Southern mapping.** To map the second locus suggested by the doublet from the PCR of C57BL/6J DNA, a 0.5 kb probe from the 3'UTR of the murine *Sybl1* cDNA was obtained by digestion with *EcoRI* and *StyI*. After digestion with *EcoRI*, 5  $\mu$ g of mouse genomic DNA from each backcross animal in the Jackson BSS and BSB panels was electrophoresed on a 0.9% agarose gel overnight. The DNAs were blotted onto HybondN nylon membrane (Amersham) by standard capillary transfer. Then, 25 ng of the fragment of *Sybl1* cDNA was labelled with [ $\alpha$ -<sup>32</sup>P]dCTP by RediPrime kit (Amersham). Hybridization was performed by standard methods (28). Stringent washing conditions were 20 min at 65°C in 0.1 $\times$  SSC, 0.1% SDS solution.

### In situ experiments

Human metaphase spreads were obtained from phytohaemagglutinin (PHA)-stimulated peripheral blood lymphocytes from a normal human donor. Metaphase spreads from primates were obtained from lymphoblastoid cell lines of chimpanzee (*Pan troglodytes*, PTR), gibbon (*Hylobates lar*, HLA), gorilla (*Gorilla gorilla*, GGO) and macaque (*Macaca fascicularis*, MFA). Metaphase spreads from a female rat-kangaroo (*Potorous tridactylis apicalis*) were obtained from PtK1 kidney cell line (ATCC number: CCL-35).

Mouse metaphases were obtained from the mouse cell line WP-G5194, kindly donated by Dr H. Hameister. This cell line (*Mus musculus domesticus*) contains a specific Robertsonian translocation, allowing easier identification of mouse chromosomes [the X chromosome and the small chromosome 19 are the only acrocentric chromosomes (29)]. Chromosome preparations were hybridized *in situ* with probes labelled with biotin by nick translation, essentially as described in (20), with minor modifications.

Briefly, 200 ng of labelled probe were used for each experiment; hybridization was performed at 37°C in 2 $\times$  SSC, 50% (v/v) formamide, 10% (w/v) dextran sulphate, 5 mg of COT-1 DNA (Boehringer Mannheim) and 3 mg of sonicated salmon sperm DNA, in a volume of 10 ml. Post-hybridization washing was at 42°C in 2 $\times$  SSC–50% formamide ( $\times$ 3) followed by three washes in 0.1 $\times$  SSC at 60°C. Biotin-labelled DNA was detected with Cy3-conjugated avidin (Amersham). Chromosome identification was obtained by simultaneous 4',6'-diamidino-2-phenylindole (DAPI) staining, which produces a Q-banding pattern.

Digital images were obtained using a Leica DMRXA epifluorescence microscope equipped with a cooled CCD camera (Princeton Instruments, NJ). Cy3 and DAPI fluorescence, detected using specific filters, were recorded separately as grey scale images. The filter set used allows capturing of fluorescence signals without any image shifting. Pseudocolouring and merging of images were performed using the Adobe Photoshop software.

## ACKNOWLEDGEMENTS

The authors gratefully acknowledge Professor R. Dulbecco for critical reading of the manuscript, Drs G. Persico, M. Di Giulio and S. Mumm for helpful comments, Drs T. Featherstone, F. Gianfrancesco, T. Esposito and M. Chirazzi for help in the initial part of the work, Mrs M. and Mr A. Terracciano for their technical assistance and C. Bouchcinsky for her secretarial assistance. Also to the memory of G. Blasi for his continuing assistance and to whom this manuscript is dedicated. This work is supported by grants from Telethon-Italy (E.526) and EC contract BMH4-CT96-1134 to M.D'U. and by grants from the Italian Association for Cancer Research (AIRC) and from Telethon-Italy to M.R. N.A.Q. is funded by a TMR post-doctoral fellowship (ERBFMBICT960649) from the EU. L.B.R. and The Jackson Laboratory interspecific backcross mapping resource are supported by a grant from the NCHGR (HG00941). This work was also supported in part by a NIH (USA) grant (HD32243) to M.S.H.K. and HG00247 to D.S.

## REFERENCES

- Graves, J.A.M. (1995) The origin and function of the mammalian Y chromosome and Y-borne genes—an evolving understanding. *BioEssays*, **17**, 311–321.
- Affara, N.A. and Ferguson-Smith, M.A. (1994) In *Molecular Genetics of Sex Determination*. Academic Press, pp. 225–265
- Bickmore, W.A. and Cooke, H.J. (1987) Evolution of homologous sequences on the human X and Y chromosomes, outside the meiotic pairing segment. *Nucleic Acids Res.*, **15**, 6261–6271.
- Chandley, A.C., Goetz, P., Hargreave, J.B., Joseph, A.M. and Speed, R.M. (1984) On the nature and extent of the XY pairing at meiotic prophase in man. *Cytogenet. Cell. Genet.*, **38**, 241.
- Burgoyne, P.S. (1982) Genetic homology and crossing over in the X and Y chromosomes of the mammals. *Hum. Genet.*, **61**, 85–90.
- Rouyer, F., Simmler, M.C., Johnsson, C., Vergnaud, G., Cooke, H.J. and Weissenbach, J. (1987) A gradient of sex linkage in the pseudoautosomal region of the human sex chromosomes. *Nature*, **319**, 291–295.
- Ohno, S. (1967) *Sex Chromosome and Sex Linked Genes*. Springer, Berlin, Heidelberg, New York.
- Disteche, C., Brannan, D.I., Larsen, A., Adler, D.A., Schorderet, D.F., Gearing, D., Copeland, N. G., Jenkins, N. A. and Park, L. S. (1992) The human pseudoautosomal GM-CSF receptor  $\alpha$  subunit is autosomal in mouse. *Nature Genet.*, **1**, 333–336.
- Miyajima, I., Levitt, L., Hara, T., Bedell, M.A., Copeland, N.G., Jenkins, N.A. and Miyajima, A. (1995) The murine interleukin-3 receptor  $\alpha$  subunit gene: chromosomal localization, genomic structure and promoter function. *Blood*, **85**, 1246–1253.
- Rappold, G.A. (1993) The pseudoautosomal region of the human sex chromosomes. *Hum. Genet.*, **92**, 315–324.
- Freije, D., Helms, C., Watson, M.S. and Donis-Keller, H. (1992) Identification of a second pseudoautosomal region near the Xq and Yq telomeres. *Science*, **258**, 1784–1787.
- Kvaloy, K., Galvagni, F. and Brown, W.R.A. (1994) The sequence organization of the long arm pseudoautosomal region of the human sex chromosomes. *Hum. Mol. Genet.*, **3**, 771–778.
- Freije, D. and Schlessinger, D. (1992) A 1.6-Mb contig of yeast artificial chromosomes around the human factor VIII gene reveals three regions homologous to probes for the *DXS115* locus and two for the *DXYS64* locus. *Am. J. Hum. Genet.*, **51**, 66–80.
- Kermouni, A., Van Roost E., Arden, K.C., Vermeesch, J.R., Weiss, S., Godelaine, D., Flint, J., Lurquin, C., Szikora, J.P., Higgs, D.R., Marynen, P. and Renaud, J.C. (1995) The IL9R receptor gene (IL9R): genomic structure and chromosomal localization in the pseudoautosomal region of the long arm of the sex chromosomes, and identification of IL9R pseudogenes at 9qter, 10pter, 16pter and 18pter. *Genomics*, **29**, 371–382.
- D'Esposito, M., Ciccodicola, A., Gianfrancesco, F., Esposito, T., Flagiello, L., Mazzarella, R., Schlessinger, D. and D'Urso, M. (1995) A synaptobrevin like gene in Xq28 pseudoautosomal region undergoes X inactivation. *Nature Genet.*, **13**, 227–229.
- Veermesch, J.R., Petit, P., Kermouni, A., Renaud, J.-C., Van Den Berghe, H. and Marynen, P. (1997) The IL9R receptor gene, located in Xq/Yq pseudoautosomal region, has an autosomal origin, escapes X inactivation and is expressed from the Y. *Hum. Mol. Genet.*, **6**, 1–8.
- Graves, J.A.M. and Watson, J.M. (1991) Mammalian sex chromosomes: evolution of organization and function. *Chromosoma*, **101**, 63–68.
- Altschul, S.F., Gish, W., Miller, W., Myers, E.W. and Lipman, D.J. (1990) Basic local alignment search tool. *J. Mol. Biol.*, **215**, 403–410.
- Shaw, M.W. and Krooth, R.S. (1964) The chromosomes of tasmanian rat-kangaroo (*Potorous tridactylis apicalis*). *Cytogenetics*, **3**, 19–33.
- Lichter, P., Tang, C. J., Call, K., Hermanson, G., Evans, G. A., Housman, D. and Ward, D. C. (1990) High-resolution mapping of human chromosome 11 by *in situ* hybridization with cosmid clones. *Science*, **247**, 64–69.
- Koop, B.F., Goodman, M., Xu, P., Chan, K. and Slightom, J.L. (1986) Primate  $\eta$ -globin DNA sequences and man's place among the great apes. *Nature*, **319**, 234–238.
- Lin, L. and Hamer, D.H. (1995) Recombination and allelic association in the Xq/Yq homology region. *Hum. Mol. Genet.*, **4**, 2013–2016.
- Disteche, C.M. (1995) Escape from X inactivation in human and mouse. *Trends Genet.*, **11**, 17–22.
- Riggs, A.D. and Pfeifer, G.D. (1992) X-chromosome inactivation and cell memory. *Trends Genet.*, **8**, 169–174.
- Scott Hansen, R., Canfield, T.C., Fjeld, A.D. and Gartler, S.M. (1996) Role of late replication in the silencing of X-linked genes. *Hum. Mol. Genet.*, **5**, 1345–1353.
- Jeppesen, P. and Turner, B.M. (1993) The inactive X chromosome in female mammals is distinguished by a lack of histone H4 acetylation, a cytogenetic marker for gene expression. *Cell*, **74**, 281–289.
- Rowe, L.B., Nadeau, J.H., Turner, R., Frankel, W.N., Letts, V.A., Eppig, J.T., Ko, M.S.H., Thurston, S.J. and Birkenmeier, E.H. (1994) Maps from two interspecific backcross DNA panels available as a community genetic mapping resource. *Mamm. Genome*, **5**, 253–274.
- Yotsumoto, S., Fujiwara, H., Horton, J.H., Mosby, T.A., Wang, X., Cui, Y. and Ko, M.S.H. (1996) Cloning and expression analysis of mouse dystroglycan gene: specific expression in maternal decidua at the peri-implantation stage. *Hum. Mol. Genet.*, **5**, 1259–1267.
- Zornig, M., Klett, C., Lovec, H., Hameister, H., Winking, H., Adolph, S. and Moroy, T. (1995) Establishment of permanent wild-mouse cell lines with readily identifiable marker chromosomes. *Cytogenet. Cell Genet.*, **71**, 37–40.
- Arveiler B., Vincent A. and Mandel J.L. (1989) Toward a physical map of a Xq28 region in man: linking color vision, G6PD, and coagulation factor an X–Y homology region. *Genomics*, **4**, 460–471.
- Brown, W.R.A., McKinnon, P.J., Villasanta, A., Spurr, N., Buckle, V.J. and Dobson, M.J. (1990) Structure and polymorphisms of human telomere-associated DNA. *Cell*, **63**, 119–132.