*Proc. Natl. Acad. Sci. USA*
Vol. 84, pp. 4954–4958, July 1987
Genetics

# Cytogenetic and molecular studies on a recombinant human X chromosome: Implications for the spreading of X chromosome inactivation

(pericentric inversion/duplication–deletion of the X chromosome/dosage blots/noninactivation/X chromosome inactivation center)

T. MOHANDAS*†, ROBIN L. GELLER*, PAULINE H. YEN*†‡, JENNIFER ROSENDORFF§, RENEE BERNSTEIN§, AKIRA YOSHIDA¶, AND LARRY J. SHAPIRO*†‡‖

*Division of Medical Genetics, Departments of †Pediatrics, and ‖Biological Chemistry, and ‡Howard Hughes Medical Institute, University of California, Los Angeles School of Medicine, Harbor/UCLA Medical Center, Torrance, CA 90509; §Department of Human Genetics, The South African Institute for Medical Research, Johannesburg 2000, South Africa; and ¶Department of Biochemical Genetics, Beckman Research Institute of the City of Hope, Duarte, CA 91010

*Communicated by Dan L. Lindsley, March 23, 1987 (received for review October 17, 1986)*

**ABSTRACT** **A pericentric inversion of a human X chromosome and a recombinant X chromosome [rec(X)] derived from crossing-over within the inversion was identified in a family. The rec(X) had a duplication of the segment Xq26.3→Xqter and a deletion of Xp22.3→Xpter and was interpreted to be Xqter→Xq26.3::Xp22.3→Xqter. To characterize the rec(X) chromosome, dosage blots were done on genomic DNA from carriers of this rearranged X chromosome using a number of X chromosome probes. Results showed that anonymous sequences from the distal end of the long arm to which probes 4D8, Hx120A, DX13, and St14 bind as well as the locus for glucose-6-phosphate dehydrogenase (*G6PD*) were duplicated on the rec(X). Mouse–human cell hybrids were constructed that retained the rec(X) in the active or inactive state. Analyses of these hybrid clones for markers from the distal short arm of the X chromosome showed that the rec(X) retained the loci for steroid sulfatase (*STS*) and the cell surface antigen 12E7 (*MIC2*); but not the pseudoautosomal sequence 113D. These molecular studies confirm that the rec(X) is a duplication–deficiency chromosome as expected. In the inactive state in cell hybrids, *STS* and *MIC2* (which usually escape X chromosome inactivation) were expressed from the rec(X), whereas *G6PD* was not. Therefore, in the rec(X) X chromosome inactivation has spread through *STS* and *MIC2* leaving these loci unaffected and has inactivated *G6PD* in the absence of an inactivation center in the q26.3→qter region of the human X chromosome. The mechanism of spreading of inactivation appears to operate in a sequence-specific fashion. Alternatively, *STS* and *MIC2* may have undergone inactivation initially but could not be maintained in an inactive state.**

Mammalian X chromosome inactivation has been intensively studied. Models for the mechanism of X chromosome inactivation must account for the initiation, chromosomal spreading, and maintenance of this regulatory phenomenon. The maintenance of X chromosome inactivation is mediated by DNA modification (1–5); however, much remains to be learned about the initiation and spreading steps in the inactivation of the mammalian X chromosome. Early studies of the Cattanach translocation (6), in which there is inactivation of X chromosome material on both sides of an inserted autosomal segment, support the concept of at least two inactivation centers (7). More extensive studies in mice carrying X chromosome-autosome translocations have shown that only a single product of the translocation undergoes inactivation (8–10). These results favor a single inactivation center on the murine X chromosome from which inactivation spreads in both directions. Studies of human X chromosome-autosome translocations also indicate a single inactivation center on the proximal long arm of the X chromosome (11). There is no convincing evidence for the inactivation of a rearranged X chromosome, lacking this segment of the human X chromosome (11–13). Three gene loci, *STS* (which codes for the enzyme steroid sulfatase), *MIC2* (which codes for the 12E7 cell surface antigen), and *XG* (which codes for the erythrocyte surface antigen Xg) located at the distal end of the short arm of the human X chromosome, escape the usual process of X chromosome inactivation (reviewed in ref. 14). Of these loci, *MIC2* has a functional homologue on the Y chromosome (15). Also present on the distal short arms of the human X and Y chromosomes are several nonexpressed pseudoautosomal sequences (16–18).

Structural aberrations of the X chromosome are useful tools for understanding the mechanisms of X chromosome inactivation. We have identified a recombinant human X chromosome [rec(X)], derived from crossing-over within a pericentric inversion of the X chromosome [inv(X)]. Cytogenetic and molecular studies on these aberrant X chromosomes are presented here, and their implications for the mechanism of spreading of X chromosome inactivation are discussed.

## MATERIALS AND METHODS

**Subjects.** A rec(X) (Fig. 1*A*) was first identified in a boy (DR) who was evaluated for severe physical and mental retardation. Proband DR had a 46,Xp+Y karyotype as there was extra material on the short arm of his X chromosome. The karyotype of his mother (Mrs. R) was interpreted as 46,X,inv(X)(p22.3q26.3) (Fig. 1*A*). The rec(X) of the proband was the product of exchange between the inverted segment of the X chromosome and its normal homologue during maternal meiosis. His karyotype was interpreted as 46,rec(X),(Xqter→Xq26.3::Xp22.3→Xqter)mat,Y (Fig. 1*A*). The rec(X) has duplication of the segment Xq26.3→Xqter and deletion of the region Xp22.3→Xpter (Fig. 1*B*). Chromosome replication studies were done in cultured lymphocytes of the mother using incorporation of 5-bromodeoxyuridine during late synthesis (19). The results revealed a random pattern of inactivation. Of 32 cells analyzed, the normal X chromosome was late replicating (and, therefore, inactive) in 7 cells, and the inv(X) was late replicating in the

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: *STS*, human gene for steroid sulfatase; *HPRT*, human gene for hypoxanthine phosphoribosyltransferase; *G6PD*, human gene for glucose-6-phosphate dehydrogenase; *MIC2*, human gene locus of a cell surface antigen detected by a monoclonal antibody, 12E7; inv(X), a pericentric inversion of the X chromosome; rec(X), recombinant X chromosome.

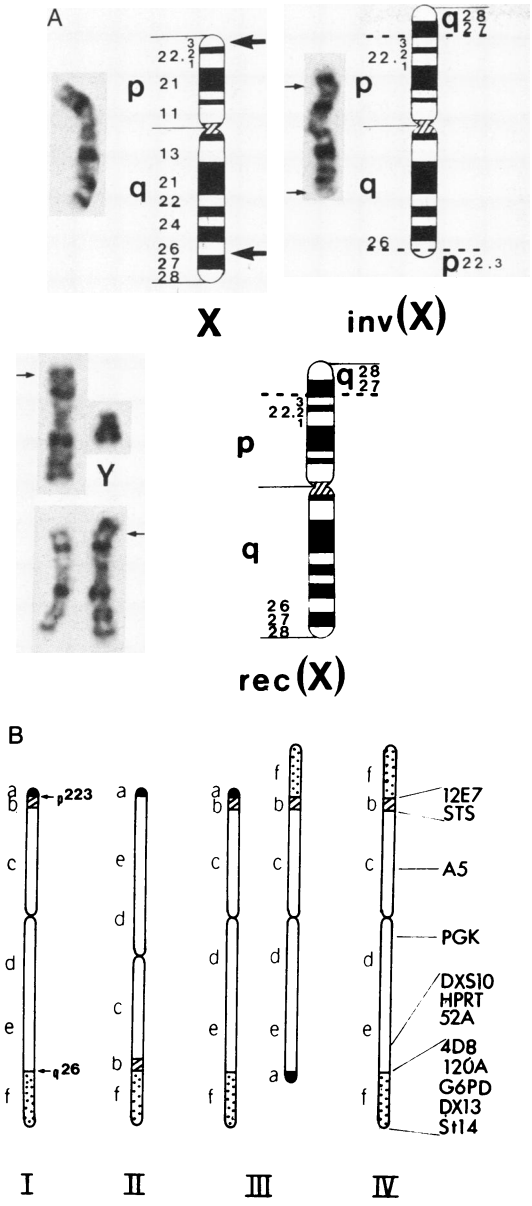


FIG. 1. (*A*) GTG-banded X and inverted X chromosomes and ideograms of the proband's mother, showing the Xp22.3 and Xq26.3 breakpoints (arrows), and the resultant rec(X) found in the male proband and his sister, showing duplication of Xq26.3→qter and deletion of Xp22.3→pter (arrows). (*B*) Diagrammatic illustration of the normal X chromosome (*I*), inverted X chromosome (*II*), pairing of the homologous regions of the X and inverted X chromosomes (*III*) presumably by formation of an inversion loop (mechanism not illustrated), and rec(X) resulting from an odd number of crossing-over events within the inverted segment (*IV*). Dark-shaded area at the distal tip of Xp (Xp22.3→Xpter) is the segment that is lost in the rec(X); striped area is the region that does not undergo inactivation (contains *MIC2* and *STS*); and the speckled area (Xq26.3→Xqter) is duplicated in the rec(X). Gene loci or anonymous sequences from the X chromosomes that were used in the study and their approximate locations are indicated on the rec(X). In addition, a pseudoautosomal sequence, 113D, was also investigated and found to be lost from the rec(X), but was retained on the inv(X) in the mother. A5 and 120A are also referred to as HxA5 and Hx120A, respectively.

remaining 25 cells. Both parents and DR were positive for the erythrocyte surface antigen Xg. Mrs. R was monitored by amniocentesis during a subsequent pregnancy, and the female fetus was found to have the same rec(X) as her brother, in addition to a normal X chromosome (Fig. 1). Replication studies in cultured amniotic fluid cells showed the rec(X) was late replicating in all 54 metaphase cells analyzed. The parents elected to continue the pregnancy and an infant girl (CR) weighing 2.6 kg was born at term. Subsequent follow-up at 2 years of age has revealed that CR has short stature. She has no other somatic feature of Turner syndrome, and her developmental milestones have been normal.

**Cell Lines.** Fibroblast cultures were established from DR, CR, and Mrs. R. Fibroblasts of DR were fused with mouse A9 cells deficient in hypoxanthine phosphoribosyltransferase (*HPRT*), and two independent hybrid clones, 110-2 and 110-8, were isolated in hypoxanthine/aminopterin/thymidine (HAT) medium containing ouabain as described (20). Fibroblasts from CR were fused with mouse B82 cells deficient in thymidine kinase (*Tk*), and two independent clones, 113-5 and 113-8-H, were isolated that retained the inactive rec(X) (nonselectively) in 53% and 93% of analyzed cells, respectively. Fibroblasts from Mrs. R were fused with A9, and clones were isolated in HAT medium. Of 10 independent clones analyzed, 9 clones contained the normal X chromosome only, and 1 clone (123-9) was found to contain the normal X chromosome and the inv(X). This clone was grown in medium containing 6-thioguanine to select against the active X chromosome. The 6-thioguanine-resistant clones were found to contain the inv(X) in a low proportion of cells. One of these clones was treated with 5-azacytidine to reactivate human *HPRT* on the inv(X), and clones were selected in HAT medium as described (21). Six of these clones analyzed cytogenetically retained the inv(X) in all cells and were evaluated for the expression of X chromosome linked markers.

Cytogenetic analysis of hybrid clones was done with the aid of Q-banding (43), and at least 10 photographed metaphases were analyzed per clone to ensure that there were no detectable chromosome rearrangements and that the chromosome of interest was intact. An additional 20 cells were examined under the microscope to further verify the human sex chromosome constitution of the hybrid clones.

**Probes and Filter Hybridization.** A total of 10 probes specifying anonymous sequences or functional genes from the human X chromosome were used. The DX numbers (22) of these probes, where known, are indicated in parentheses. Probe HxA5 (DXS187) detects an anonymous, unique, X chromosome-specific sequence from the region Xp11.1→Xp22.1 (23). Probes DXS10 (24), 52A (DXS51) (25), 4D8 (DXS98) (24), Hx120A (23), DX13 (DXS15) (25), and St14 (DXS52) (26) recognize single-copy sequences from the Xq26→qter region of the human X chromosome. Probe 113D (DXYS15) detects an anonymous pseudoautosomal sequence from the distal end of the short arm of the X chromosome (and also the Y chromosome) (16). The HPRT probe consisted of a 0.6-kilobase (kb) *Msp* I fragment from the first intron of the human *HPRT* gene (27, 28). To detect the glucose-6-phosphate dehydrogenase (*G6PD*) locus, a G6PD cDNA clone, G6PD-19 (29), or a 1.6-kb genomic probe specific for X chromosome-linked *G6PD* (A.Y., unpublished data) was used as the hybridization probe. A cDNA probe for human cytosolic aldehyde dehydrogenase 1 (*ALDH1*) located on human chromosome 9 was used as an internal control (30, 31).

DNA was extracted from fibroblasts or leukocytes of controls, DR, CR, Mrs. R, and different hybrid clones as described (23). To determine by dosage the number of copies of probes HxA5, DXS10, 52A, 4D8, Hx120A, DX13, St14, and HPRT, high molecular weight DNA (5 μg) was digested with a suitable restriction enzyme, electrophoresed on agarose gels, and transferred to nitrocellulose filters (32). Probe HxA5 from the proximal short arm of the X chromosome was used as an internal reference for the amount of DNA present on nitrocellulose filters. Different restriction enzymes were used for different probes so that fragments

hybridizing to probe HxA5 were well separated from fragments hybridizing to the test probe. Probes were labeled by nick-translation, and hybridization was carried out as described (23). The resulting bands were quantitated by densitometry of the autoradiograms. For Southern blot analysis using 113D, genomic DNA (5 μg) was digested with *Hin*dIII and hybridized to $^{32}$P-labeled 113D. After autoradiography, the nitrocellulose filters were stripped of the probe (33) and rehybridized with HxA5. To detect the *G6PD* and autosomal *ALDH1* loci, the probes were labeled by nick-translation with [$^{32}$P]dATP (5000 Ci/mmol; 1 Ci = 37 GBq) and hybridized to ≈5 μg of genomic DNA digested with *Msp* I or *Pst* I. Filters were first hybridized to the G6PD probe, washed, and then rehybridized with the ALDH1 probe. The number of *G6PD* loci in DR and in CR were estimated by densitometry of the autoradiograms.

**X Chromosome Linked Markers.** Expression of the X chromosome linked markers *STS* (34, 35), 12E7 (36, 37), and *G6PD* (38) was evaluated in cultured cells.

## RESULTS

Cytogenetic studies indicated that rec(X) has a duplication of the segment Xq26.3→Xqter (Fig. 1). Deletion of any material from the distal short arm of the X chromosome was too small to be detected by light microscopy. To test the validity of the cytogenetic findings, Southern blots were done on genomic DNA from DR, CR, and Mrs. R, as well as control males and females, using a number of different probes from the X chromosome and evaluated for dosage. Quantitation of the hybridization intensities of resulting bands was done using densitometry.

Genomic DNA from the control female IMR90, the control males IMR91 and ERW, the proband (DR) with the rec(X) and a Y chromosome, and the sister (CR) of the proband with an X chromosome and the rec(X) were digested with a suitable restriction enzyme and hybridized to probes HxA5, DXS10, 52A, 4D8, Hx120A, DX13, St14, and HPRT. Probe HxA5 detects an anonymous, unique sequence from the proximal short arm of the X chromosome and was used as an internal reference for the amount of DNA present on the nitrocellulose filters. Copy numbers of the sequences to which the various probes bind were estimated by quantitative densitometry of the autoradiograms except for probe *DXS10* that had a high background (Table 1). Results presented in Fig. 2 and Table 1 show that probes HxA5, DXS10, HPRT, and 52A bind to sequences that are present in single copies (normal male level) in patient DR whereas probes 4D8, Hx120A, DX13, and St14 bind to sequences that are present in two copies (normal female level). In the sister of the proband, probes HxA5, DXS10, HPRT, and 52A bind to sequences that are present in two copies, whereas probes 4D8, Hx120A, DX13, and St14 bind to sequences that are present in three copies.

The number of copies of *G6PD* was of particular interest, as we suspected that *G6PD* might be duplicated on the rec(X) based on the cytogenetic findings. Also the expression of *G6PD* could be evaluated from the inactive rec(X). A cDNA probe for human *G6PD* or a genomic probe specific for X chromosome-linked *G6PD* was used to determine the number of *G6PD* loci in DR and CR. In *Pst* I-digested human DNA, the G6PD cDNA probe detects four bands at 5 kb, 4 kb, 2 kb, and 0.6 kb. Of these, the 4-kb band is derived from sequences located on human chromosome 17 (39). Therefore, the 4-kb band was used as an internal reference for quantitation of the X chromosome-linked 2-kb band in the DNA of DR and CR. The results indicated that DR and CR have two and three *G6PD* loci, respectively, in their genomic DNA (data not shown). The 1.6-kb genomic probe detects only X chromosome-linked *G6PD* and was employed as a probe on *Msp* I-digested genomic DNA from DR, CR, and controls. A cDNA probe for autosomal *ALDH1* was used as an internal reference for quantitation of DNA on the nitrocellulose filters. Data shown in Fig. 3 and Table 1 indicate that DR and CR have two and three copies of *G6PD*, respectively.

Table 1. Densitometric quantitation of hybridization bands

| DNA probe | Relative intensities of hybridization: Female IMR90 | Male IMR91 | Male ERW | Male DR | Male CR |
|---|---|---|---|---|---|
| HxA5* | (2.00) | (1.00) | (1.00) | (1.00) | (2.00) |
| HPRT* | 2.00 | 0.72 | 1.12 | 1.01 | 1.92 |
| 52A* | 2.00 | 0.79 | 1.32 | 0.89 | 1.81 |
| 4D8* | 2.00 | 0.81 | 1.10 | 2.03 | 2.67 |
| Hx120A* | 2.00 | 1.17 | 1.44 | 1.95 | 2.82 |
| DX13* | 2.00 | 0.75 | 0.97 | 2.08 | 3.23 |
| St14* | 2.00 | 1.04 | 1.17 | 2.03 | 2.63 |
| ALDH1† | (2.00) | (2.00) | | (2.00) | (2.00) |
| G6PD† | 2.00 | 1.18 | | 2.15 | 3.28 |

The relative intensity of the signals in Figs. 2(*) and 3(†) were measured with a densitometer. Values shown indicate the copy number of the loci to which each probe binds and were normalized taking the signals given by the female control as 2.00 in parentheses. The HxA5 signal was used as the internal reference for intensities from Fig. 2. Two copies of HxA5 sequences are present in the female control and CR, and one copy of HxA5 is present in male controls and DR. A probe for autosomal *ALDH1* was used as the internal reference for intensities from Fig. 3.

The dosage studies clearly indicate that the rec(X) contains a duplication of material from the distal long arm of the X chromosome that includes *G6PD*. We sought to determine if the rec(X) has a deletion of the distal end of the short arm of the X chromosome, by evaluating the presence and expression of markers from this region. Proband DR did not have ichthyosis, a skin disorder found in patients who lack STS enzymatic activity (14). Fibroblasts of DR expressed STS (Table 2), as expected, and since there is no functional gene for STS on the Y chromosome, *STS* must be present on the rec(X). Using X/Y chromosomal translocations, we (37) and others (40) have shown that *MIC2* maps distal to *STS* on the short arm of the X chromosome. We determined the expression of 12E7 (*MIC2*) from the rec(X), using mouse–human

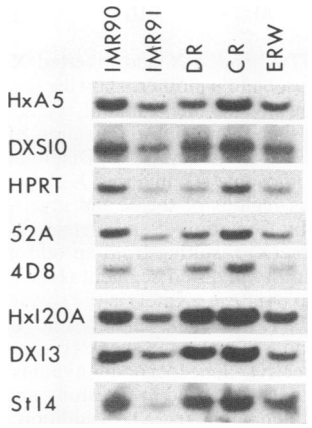


FIG. 2. Southern blot analysis of genomic DNA from control female (lane IMR90), males (lanes IMR91 and ERW), proband (lane DR) with the rec(X), and the proband's sister (lane CR) with an X and the rec(X), using various X chromosome probes. The resulting bands were quantitated (Table 1) by densitometry of the autoradiograms. Intensities of the hybridizing bands show that loci to which probes HxA5, DXS10, HPRT, and 52A bind are not duplicated in DR, whereas loci to which probes 4D8, Hx120A, DX13, and St14 bind are duplicated (intensity comparable to the female control). The figure is a composite of autoradiograms.

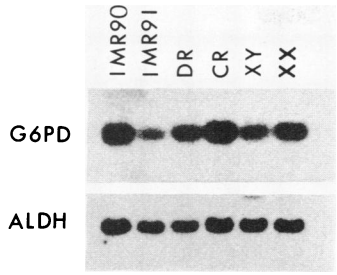


FIG. 3. Southern blot analysis of genomic DNA from control females (lanes IMR90 and XX), control males (lanes IMR91 and XY), proband (lane DR), and his sister (lane CR) using a genomic probe for human X chromosome-linked *G6PD* and a cDNA probe for ALDH1 (ALDH) coded autosomally. DNA (5 μg) was digested with *Msp* I and hybridized to the G6PD probe. Following autoradiography, the probe was "melted off" the filter, and then the filter was rehybridized to the ALDH probe. The ALDH1 cDNA 1 probe detects multiple bands in human DNA following *Msp* I digestion, some of which are polymorphic; a constant band is shown here and was used for densitometry. Control males, IMR91, and DR, have approximately the same amount of DNA based on ALDH1 signal. However, the signal is more intense in DR with the G6PD probe indicating two loci for *G6PD* in his genomic DNA.

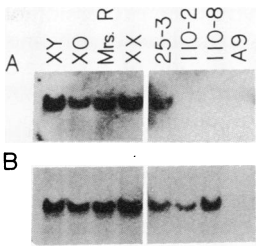


FIG. 4. Southern blot analysis using the pseudoautosomal sequence 113D (DXYS15). Genomic DNA was isolated from male (lane XY) and female (lane XX) controls, a patient with the Turner syndrome (lane XO), Mrs. R, a mouse–human hybrid cell line containing a normal human X chromosome (lane 25-3), two mouse–human hybrids containing the rec(X) from DR (lanes 110-2 and 110-8), and the mouse parental line for the hybrids (lane A9). About 5 μg of DNA was digested with *Hin*dIII and hybridized with $^{32}$P-labeled 113D (*A*). After autoradiography, the nitrocellulose filters were stripped of the hybridization probe and rehybridized with probe HxA5 (*B*). The results show that the rec(X) in DR has lost 113D sequences, whereas the mother of DR (Mrs. R) has 113D sequences on the inv(X) as the intensity of hybridization in her DNA is comparable to XY and XX controls.

cell hybrids derived from the fusion of mouse A9 cells and fibroblasts from DR. Results from two independent hybrid clones (110-2 and 110-8) containing rec(X), but not a Y chromosome (Table 2), show that 12E7 is expressed from rec(X). In addition, DR is positive for the expression of *XG*, for which there is no functional homologue on the Y chromosome. Thus the rec(X) contains *STS*, *MIC2*, and *XG*, and the breakpoint in the inversion is distal to these loci. A probe for the more distal pseudoautosomal region shared by X and Y chromosomes, 113D, was also employed to detect any deletion of X material in the rec(X). The results presented in Fig. 4 show that mouse–human cell hybrid clones containing the rec(X) from DR have lost 113D sequences. However, the intensity of the signal in Mrs. R is comparable to that in genomic DNA from normal XX and XY individuals indicating that the inv(X) has retained 113D sequences. In addition, analysis of genomic DNA from a hybrid clone (123-9) retaining the partially active inv(X) showed it to be positive for 113D sequences (data not shown). The results, therefore, show that rec(X) is truly a duplication–deficiency chromosome expected to result from crossing-over in a heterozygote for a pericentric inversion. A diagram of this chromosome is presented in Fig. 1*B*.

To determine the expression of X chromosome-linked markers from the inactive rec(X), cell hybrids were constructed from the fusion of mouse cells and fibroblasts from CR, in whom the rec(X) is inactive. Multiple independent clones were screened for rec(X), and two were identified (113-5 and 113-8-H) that retained the inactive rec(X) nonselectively in 53% and 93% of cytogenetically analyzed cells, respectively. These two clones expressed human STS, but not human G6PD (Table 2). Clone 113-5 was also tested for the expression of 12E7 and was found to be positive. These results show that both *G6PD* loci on the rec(X) have been inactivated whereas *STS* and *MIC2* have not undergone inactivation. Thus there is inactivation of X-chromosome material on both sides of the segment that escapes X chromosome inactivation. Evaluation of hybrid clones retaining the partially active (following 5-azacytidine treatment) inv(X) from Mrs. R showed that they expressed human STS but not G6PD.

Table 2. Expression of human X chromosome-linked markers in cultured cells

| Cell strain/ clone | Human sex chromosome constitution | Expression of human gene* | | |
|---|---|---|---|---|
| | | *STS* | *MIC2* | *G6PD* |
| DR fibroblasts | rec(X),Y | + | ND | + |
| 110-2 | Active rec(X) | ND | + | + |
| 110-8 | Active rec(X) | ND | + | ND |
| 113-5 | Inactive rec(X) | + | + | − |
| 113-8-H | Inactive rec(X) | + | ND | − |
| 123-9 | Partially active inv(X) | + | ND | − |

*+ indicates presence and − indicates absence of the human marker. ND, not done.

## DISCUSSION

An inv(X) and a rec(X) derived from crossing-over within this inversion were identified in a family. The cytogenetic and molecular studies presented show that the rec(X) contains duplication of q26.3→qter of the X chromosome and deletion of p22.3→pter of the X chromosome (Fig. 1). Results of the dosage studies presented are consistent with the known linkage relationships and order of genes in the distal Xq region. Using restriction fragment length polymorphisms, it has been shown that the probe DXS10 binds to sequences that are as closely linked to *HPRT* as are the sequences to which 52A binds (24). These loci form a cluster that is removed by ≈30 centimorgans, from a more distal cluster that includes *G6PD*, and sequences to which probes DX13, and St14 bind. Thus the breakpoint on Xq leading to the production of the inv(X) appears to be located between the *HPRT* and *G6PD* clusters of loci.

Studies on the structurally abnormal human X chromosomes presented here are interesting from several standpoints. They confirm, by molecular genetic techniques, the production of a duplication–deficiency chromosome as a result of crossing-over within a pericentric inversion. Further, these results show that loci such as *STS* and *MIC2* can be expressed from inactive structurally abnormal human X chromosomes. It had been suggested that *XG* may be inactivated on structurally abnormal human X chromosomes (41), and we had shown (42) that *STS* and *MIC2* can be expressed from deleted, inactive human X chromosomes. The results presented here extend these findings and show expression of *STS* from the inactive inv(X) and rec(X).

More interestingly, evaluation of gene expression from the inactive rec(X) shows that, whereas it continues to express

*STS* and *MIC2*, there is no expression of *G6PD* from either of the duplicated loci. This clearly indicates that on this rec(X) there is inactivation of X chromosome material separated by the region containing *STS* and *MIC2*. As noted earlier, there is no convincing evidence for an inactivation center in the q26.3→qter region of the human X chromosome. Therefore, the mechanism for spreading of inactivation can leave loci that escape inactivation unaffected and proceed to inactivate distal loci that normally undergo inactivation.

Two additional arguments can be made to support the premise that there is inactivation of X chromosome material separated by *STS* and *MIC2*. First, rec(X) is inactivated in CR, who is phenotypically normal other than having short stature. If inactivation had not spread to the q26.3→qter region, her phenotype might be expected to be similar to that of her brother DR as she would also have two active copies of the q26.3→qter region of the X chromosome. Secondly, in Mrs. R, the inv(X) is inactive in the majority of lymphocytes analyzed. Based on the molecular and enzyme marker studies on the rec(X), it is apparent that in the inv(X) the region containing *STS*, *MIC2*, and *XG* is bracketed by X-chromosome material that undergoes inactivation (Fig. 1*B*). If inactivation had not spread into the q26.3→qter region on the inv(X), she would also have two active copies of this region, and one would expect the cells in which the inv(X) is inactive to be selected against. Hybrid clones retaining the inactive inv(X) from Mrs. R expressed *STS* but not *G6PD*, indicating that inactivation has in fact spread into the q26.3→qter region on this chromosome.

The results presented here suggest that the mechanism of spreading of X chromosome inactivation may operate in a sequence-specific fashion. Loci such as *STS* that escape inactivation could lack these sequences and, therefore, not be inactivated. Alternatively, it may be that these loci are inactivated initially but cannot be maintained in the inactive state, as they lack the recognition signals for that process.

The authors are grateful to M. B. Passage and L. Rolewic for expert technical contributions and N. Hitt for assistance in preparation of this manuscript. We thank T. Caskey, R. Nussbaum, D. Drayna, J. L. Mandel, and J. Weissenbach for some of the X chromosome probes used in this study. This work was supported by Public Health Service Grants HD12178, HD17156, HL29515, and HD15193 and Grant 1-639 from The March of Dimes. T.M. is the recipient of a Research Career Development Award from the National Institute of Child Health and Human Development. L.J.S. is an Investigator and P.Y. is an Associate of the Howard Hughes Medical Institute, UCLA School of Medicine.

1. Liskay, R. M. & Evans, R. M. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 4895–4898.
2. Venolia, L. & Gartler, S. M. (1983) *Nature (London)* **302**, 82–83.
3. Chapman, V. M., Kratzer, P. G., Siracusa, L. D., Quarantillo, B. A., Evans, R. & Liskay, R. M. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 5357–5361.
4. Venolia, L., Gartler, S. M., Wassman, E. R., Yen, P., Mohandas, T. & Shapiro, L. J. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 2352–2354.
5. Lester, S. C., Korn, N. J. & DeMars, R. (1982) *Somatic Cell Mol. Genet.* **9**, 265–284.
6. Cattanach, B. M. (1970) *Genet. Res.* **16**, 293–301.
7. Eicher, E. M. (1970) *Adv. Genet.* **15**, 175–259.
8. Russell, L. B. & Cacheiro, N. L. A. (1978) in *Genetic Mosaics and Chimeras in Mammals*, ed. Russell, L. B. (Plenum, New York), pp. 393–416.
9. Takagi, N. (1980) *Chromosoma* **81**, 439–459.
10. Rastan, S. (1983) *J. Embryol. Exp. Morphol.* **78**, 1–22.
11. Therman, E. & Patau, K. (1974) *Humangenetik* **25**, 1–16.
12. Summitt, R. L., Tipton, R. E., Wilroy, R. S., Jr., Martens, P. R. & Phelan, J. P. (1978) *Birth Defects Orig. Artic. Ser.* **14(6C)**, 219–247.
13. Mattei, M. G., Mattei, J. F., Vidal, I. & Giraud, F. (1981) *Hum. Genet.* **56**, 401–408.
14. Shapiro, L. J. (1985) *Adv. Hum. Genet.* **14**, 331–381.
15. Buckle, V., Mondello, C., Darling, S., Craig, I. W. & Goodfellow, P. N. (1985) *Nature (London)* **317**, 739–741.
16. Simmler, M. C., Rouyer, F., Vergnaud, G., Nystrom-Lahti, M., Ngo, K. Y., de la Chapelle, A. & Weissenbach, J. (1985) *Nature (London)* **317**, 692–697.
17. Cooke, H. J., Brown, W. R. A. & Rappold, G. A. (1985) *Nature (London)* **317**, 687–692.
18. Rouyer, F., Simmler, M. C., Johnsson, C., Vergnaud, G., Cooke, H. J. & Weissenbach, J. (1986) *Nature (London)* **319**, 291–295.
19. Dutrillaux, B., Laurent, C., Coturier, J. & Lejeune, J. (1973) *C. R. Hebd. Seances Acad. Sci. Ser D* **276**, 179–181.
20. Mohandas, T., Heinzmann, C., Sparkes, R. S., Wasmuth, J., Edwards, P. & Lusis, A. J. (1986) *Somatic Cell Mol. Genet.* **12**, 89–94.
21. Mohandas, T., Sparkes, R. S. & Shapiro, L. J. (1981) *Science* **211**, 393–396.
22. Willard, H. F., Skolnik, M. H., Pearson, P. L. & Mandel, J. L. (1985) *Cytogenet. Cell Genet.* **40**, 360–489.
23. Yen, P., Marsh, B., Mohandas, T. K. & Shapiro, L. J. (1984) *Somatic Cell Mol. Genet.* **10**, 561–571.
24. Boggs, B. A. & Nussbaum, R. L. (1984) *Somatic Cell Mol. Genet.* **10**, 607–613.
25. Drayna, D., Davies, K., Hartley, D., Mandel, J.-L., Camerino, G., Williamson, R. & White, R. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 2836–2839.
26. Oberle, I., Drayna, D., Camerino, G., White, R. & Mandel, J. L. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 2824–2828.
27. Patel, P. I., Framson, P. E., Caskey, C. T. & Chinault, A. C. (1986) *Mol. Cell Biol.* **6**, 393–403.
28. Yen, P. H., Patel, P., Chinault, A. C., Mohandas, T. & Shapiro, L. J. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 1759–1763.
29. Takizawa, T., Huang, I.-Y., Ikuta, T. & Yoshida, A. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 4157–4161.
30. Hsu, L. C., Tani, K., Fijiyoshi, T., Kurachi, K. & Yoshida, A. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 3771–3775.
31. Hsu, L. C., Yoshida, A. & Mohandas, T. (1986) *Am. J. Hum. Genet.* **38**, 641–648.
32. Southern, E. M. (1975) *J. Mol. Biol.* **98**, 503–517.
33. Parada, L. F., Tabin, C. J., Shih, C. & Weinberg, R. A. (1982) *Nature (London)* **297**, 474–478.
34. Shapiro, L. J., Weiss, R., Buxman, N. M., Vidgoff, J., Dimond, R. L., Roller, J. A. & Wells, R. S. (1978) *Lancet* **ii**, 756–757.
35. Mohandas, T., Shapiro, L. J., Sparkes, R. S. & Sparkes, M. C. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 5779–5783.
36. Goodfellow, P., Banting, G., Levy, T., Povey, S. & McMichael, A. (1980) *Somatic Cell Genet.* **6**, 777–787.
37. Geller, R. L., Shapiro, L. J. & Mohandas, T. K. (1986) *Am. J. Hum. Genet.* **38**, 884–890.
38. Mohandas, T., Sparkes, R. S., Bishop, D. F., Desnick, R. J. & Shapiro, L. J. (1985) *Am. J. Hum. Genet.* **36**, 916–925.
39. Yoshida, A. & Lebo, R. V. (1986) *Am. J. Hum. Genet.* **39**, 203–206.
40. Ropers, H. H., Zimmer, J., Strobl, G. & Goodfellow, P. (1985) *Cytogenet. Cell Genet.* **40**, 736 (abstr.).
41. Polani, P. E., Angell, R., Giannelli, F., de la Chapelle, A., Race, R. R. & Sanger, R. (1970) *Nature (London)* **227**, 613–616.
42. Immken, L., Mohandas, T., Sparkes, R. S. & Shapiro, L. J. (1984) *Am. J. Hum. Genet.* **36**, 979–986.
43. Caspersson, T., Zech, L. & Johansson, C. (1970) *Exp. Cell Res.* **60**, 315–319.