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The Steroid Sulfatase Locus on Structurally Abnormal Inactive X Chromosomes Is Expressed

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SUMMARY

In mammalian somatic cells, sex-chromosome dosage compensation is achieved by random inactivation of one of the two X chromosomes. The Xg blood group antigen (*Xg*) and steroid sulfatase (*STS*) loci on the distal end of the short arm of the X chromosome have been shown to escape this inactivation. However, it has been reported that on structurally abnormal inactive X chromosomes *Xg* and *STS* are inactivated. This discrepancy requires further consideration since whatever process accounts for the lack of inactivation of these loci on structurally normal, inactive X chromosomes might be anticipated to be operative on structurally abnormal, inactive X chromosomes. To investigate this issue, we examined the expression of STS activity in mouse-human somatic-cell hybrids retaining two different, deleted, inactive human X chromosomes. These studies provide evidence for lack of inactivation of STS on structurally abnormal, inactive X chromosomes.

INTRODUCTION

Deficiency of steroid sulfatase (STS) is the basic defect in X-linked ichthyosis [1]. This gene has been mapped to the distal portion of the short arm of the X chromosome by somatic-cell hybridization techniques using X-autosome translocations [2] and by deletion mapping [3–5]. The *STS* locus has been shown to

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escape the usual X-inactivation [6] on structurally normal, inactive X chromosomes in fibroblast clones of individuals heterozygous at the loci for both STS and glucose-6-phosphate dehydrogenase (G6PD) [7, 8]. Lack of inactivation of STS has also been demonstrated in mouse-human somatic-cell hybrids [9]. The locus for the Xg blood group that is linked (10 cM) to *STS* is also known to escape inactivation [10], supporting the hypothesis that the distal portion of the X-chromosome short arm is not inactivated in structurally normal, inactive X chromosomes. However, there is evidence to suggest that the distal segment of the short arm may be inactivated on structurally rearranged inactive X chromosomes. Polani et al. [11] studied Xg blood groups in families of 19 patients with various deletions of the long arm of the X chromosome. They identified three female patients who were Xg(a−) with Xg(a+) fathers. As they would have obligatorily inherited their fathers' Xg^a gene, these girls should be Xg(a+). In only one of these three patients, however, could nonpaternity or mosaicism be ruled out. This girl's deleted X chromosome was shown to be late-labeling by autoradiography. Polani et al. pooled the remaining cases to compare the distribution of the Xg antigen in this group with values for normal males and females. They found that the frequency of antigen positivity in patients with long-arm deletions was similar to that of the general male population, thus suggesting that only a single copy of this gene was active. Sanger et al. [12] cataloged Xg blood groups in 1,547 patients with various abnormalities of sex-chromosome structure or number. Of 20 patients with nonmosaic long-arm deletions, 13 or 65% were Xg(a+), a value comparable to the frequency of this allele in the male population (66%) and different from that in normal females (88%), suggesting that *Xg* is inactivated on deleted inactive X chromosomes. However, *Xg* is not inactivated on all structurally abnormal, inactive X chromosomes, as it was found to be expressed from an inactive X/3 translocation (Xpter→Xq26 or Xq28::3q21→3qter) [13].

Ropers et al. [14] examined quantitative differences in the activity of STS in fibroblasts of patients with structural aberrations of the X chromosome. Two of these patients, each with two cytogenetically evident copies of distal Xp (and presumably the *STS* gene), showed late-replication of the structurally abnormal X chromosome. One had a deletion of the long arm of an X chromosome, and the other had an unbalanced X/13 translocation. These individuals had STS activities in the "male control" range. Ropers et al. concluded that the *STS* locus, like the *Xg* blood group gene, is inactivated on structurally abnormal X chromosomes. They did acknowledge, however, that there is considerable overlap not only between their male and female values, but also between their groups with one and two copies of the gene. Chance and Gartler [15] also reported that the activity of STS in one patient with a deletion of an X was comparable to their male control level and suggested that *STS* may be inactivated on structurally aberrant X chromosomes. Comparison of male and female levels of STS in different laboratories and in different tissues has consistently shown a dosage effect, which, however, is less than the expected ratio of 1:2 [8, 14–17].

To further study X-inactivation in structurally abnormal, inactive X chromosomes, we employed somatic-cell hybridization techniques and examined STS expression. Direct evidence for noninactivation of this locus would be obtained

from hybrid clones containing the structurally abnormal X chromosome that express STS activity.

MATERIALS AND METHODS

Hybrid clones were derived from the fusion of mouse B82 cells with two human lines containing different deletions of the long arm of the X chromosome. The mouse B82 parental line is deficient in STS and thymidine kinase (TK). One of the human parental lines was established in our laboratory from a skin biopsy of a patient with a terminal deletion of the long arm of the X chromosome [46,X,del(X)(q22)]. Based on the genetic map of the human X chromosome [18], this deleted X chromosome should retain the *STS* and phosphoglycerate kinase (*PGK*) loci but not the *G6PD* locus. The patient and both her parents were Xg(a+). The second human parental line (GM3923) was obtained from the Human Genetic Mutant Cell Repository (Camden, N.J.). This cell line has an interstitial deletion on the long arm of the X chromosome [46,X,del(X)(q13q22)]. This deleted X chromosome should retain the loci for hypoxanthine-guanine phosphoribosyl transferase (HPRT) and G6PD. It is uncertain whether it includes the loci for PGK and α-galactosidase (GLA). Presence of *HPRT* on this X was demonstrated by reactivation of this gene with 5-azacytidine [19] (T. M. and L. J. S., unpublished observations, 1983). Therefore, it is reasonable to assume that *G6PD* is also present on this chromosome as it is located distal to HPRT [20].

Cells were fused in monolayer using a 50% solution of polyethylene glycol (mol. wt. 1,000) in balanced salt solution containing 7% DMSO [21, 22]. Hybrid cells were selected in HAT medium [23, 24] containing ouabain [25]. Independent primary hybrid clones were isolated from separate dishes. STS activity of hybrid clones was determined as described [2, 26]. Expression of G6PD [27] and PGK [28] in cell hybrids was evaluated by electrophoresis. Replication pattern of chromosomes in hybrid clones was studied using the 5-bromodeoxyuridine (BrdU)-acridine-orange technique [29].

RESULTS AND DISCUSSION

The results of the fusion experiments are summarized in table 1. Thirty-seven independent, primary clones were obtained from the first fusion involving cells with a terminal deletion of the X. Four of these were found to be STS^+ and $G6PD^-$, thus indicating that the deleted X chromosome should be present if STS escapes X-inactivation. Three of these clones (70-46, 70-62, and 70-51) with appreciable levels of STS activity were characterized in detail biochemically and cytogenetically. All were found to contain the deleted X and no other human X-chromosomal material (fig. 1). They expressed STS, but not human G6PD or PGK. Based on our previous studies [2], it is reasonable to presume that the STS activity observed in these hybrid clones is of human origin. This was confirmed employing a species specific anti-STS monoclonal antibody (Shapiro et al., unpublished observations, 1984).

To prove that the STS activity occurred only in cells containing the deleted X chromosome, clone 70-51, which retained the del(X) in 58% of cells, was subcloned in microtest plates. Of 11 subclones that were STS^-, one was analyzed cytogenetically and was found to have lost the Xq− chromosome. Fourteen subclones were STS^+. One of these was subjected to cytogenetic analysis, and it still contained the deleted X chromosome. Thus, STS activity segregated with the abnormal X chromosome. Lack of expression of human PGK in hybrid clones 70-46, 70-62, and 70-51 suggested that the deleted X is indeed inactive. This

TABLE 1

BIOCHEMICAL AND CYTOGENETIC ANALYSES OF MOUSE-HUMAN HYBRID CLONES

HYBRID CLONE	EXPRESSION OF HUMAN			% OF ANALYZED METAPHASES WITH del(X)
	G6PD	STS	PGK	
Fusion #1				
B82 × 46,X,del(X)(q22):				
22 clones	−	−	...	...
11 clones	+	+	...	...
4 clones	−	+	...	...
Clone 70-46	−	+	−	17
Clone 70-62	−	+	−	84
Clone 70-51	−	+	−	58
11 subclones of clone 70-51	...	−	...	...
Subclone 70-51-1	...	−	...	0
14 subclones of clone 70-51	...	+	...	...
Subclone 70-51-26	...	+	...	80
Fusion #2				
B82 × 46,X,del(X)(q13q22):				
6 clones	−	−	...	...
5 clones	+	+	...	...
6 clones	−	+	...	...
Clone 77-40B	−	+	...	79
Clone 77-40B	−	+	...	77

NOTE: + indicates presence and − indicates absence of the human marker.

was further demonstrated by chromosome-replication studies using the BrdU-acridine-orange technique in clone 70-62. The deleted X chromosome did not fluoresce in cells that have incorporated BrdU at the end of S phase, indicating that it was late replicating (fig. 2). These results show that STS can be expressed from an inactive terminally deleted human X chromosome.

Seventeen independent primary clones were isolated from the second cell fusion in which the human parental cells had an interstitial deletion of the human X chromosome. Six of these hybrids were found to be STS^+ and $G6PD^-$. Two of these clones with high levels of STS expression were further analyzed. Cytogenetic analysis of 30 Q-banded metaphase photographs showed the presence of the deleted X (and no other human X material) in 77% and 79% of analyzed cells. Since *G6PD* should be on this deleted chromosome, lack of expression of this gene shows that this structurally aberrant X chromosome is indeed inactive. Expression of STS in these clones demonstrates that this locus escapes inactivation on a second deleted inactive X chromosome.

Three of the five clones analyzed in detail (70-62, 77-40b, 77-46) from the two fusion experiments were also found to be positive for the expression of the cell-surface antigen, 12E7 [30]. As the locus for 12E7 (MIC2X) has been assigned to the distal end of the short arm of the human X [31], this provides additional support for the noninactivation of this region on the two inactive, deleted, human X chromosomes.

We have demonstrated that STS can be expressed from structurally abnormal, inactive human X chromosomes in somatic-cell hybrids. The discrepancy between these results and earlier studies on Xg blood groups [11, 12] requires further

analysis. Unfortunately, it is not possible to ascertain the presence of the Xg antigen in somatic cells, making somatic-cell genetic studies infeasible at this time. Since we initially screened the hybrid clones for the expression of STS, it is possible that there were cells in the original population that retained either of these two inactive human X chromosomes but did not express STS. This would imply heterogeneity, with some inactivated chromosomes permitting STS expression, and other chromosomes being completely inactive. Alternatively, it may be that inactivation patterns are different on different structurally abnormal inactive human X chromosomes and that the two cases studied here are somehow unique. This seems unlikely.

One interesting possibility for reconciling these results is that Xg behaves like STS, which Migeon et al. [8] showed to have differential expression; that is, STS expression from inactive X chromosomes is quantitatively less than that from active X chromosomes. Xg heterozygotes have been known to give distinctly weaker agglutination reactions than hemizygotes or homozygotes [10]. If the gene for the Xg antigen was on a structurally abnormal and, therefore, preferentially inactivated X-chromosome, it is possible that it might produce levels too low to be detected. Indeed, Polani et al. [11] considered this option but dismissed it because they would have expected to see individual differences in Xg activity.

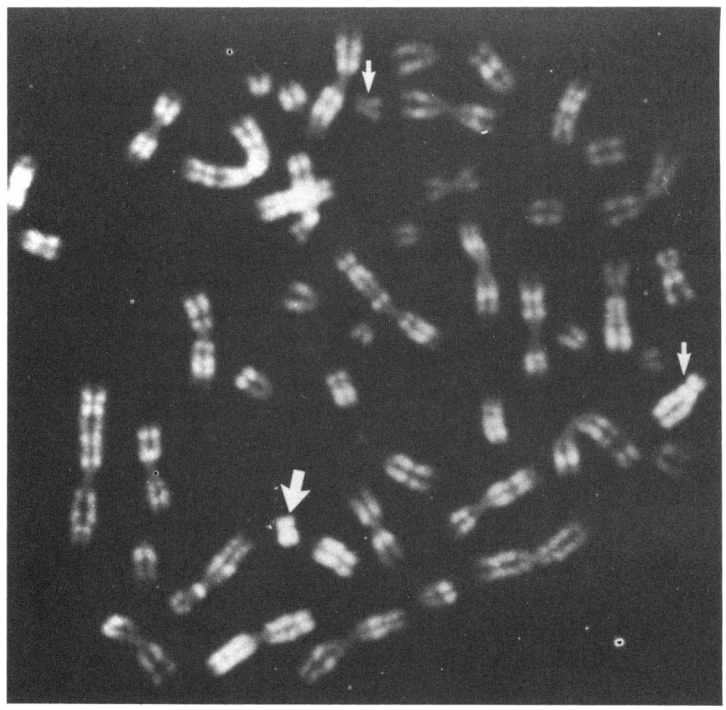

FIG. 1.—A Q-banded metaphase spread from the mouse-human hybrid clone, 70-62, containing del(X)(q22). Human chromosomes 4 and 17 (*arrows*) and del(X) (*bolder arrow*) are present in this metaphase.

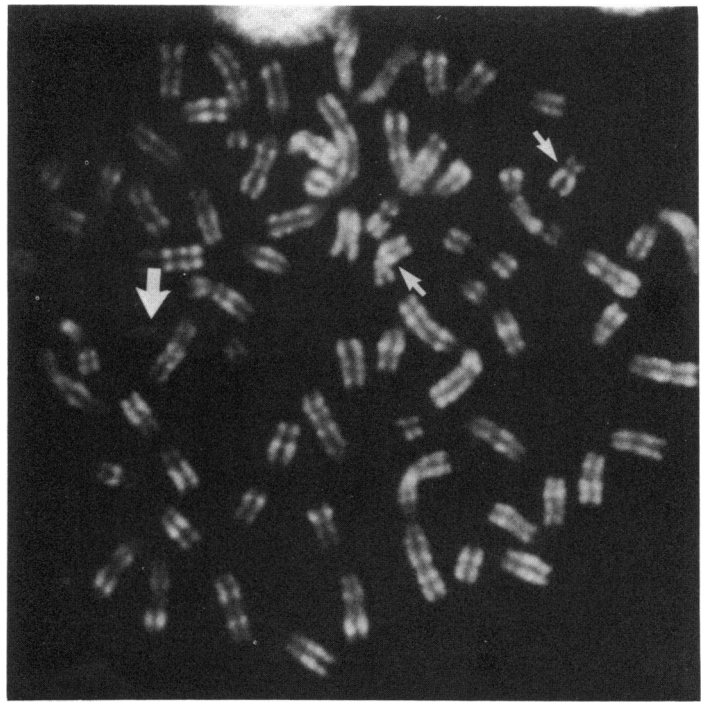

FIG. 2.—Replication pattern of chromosomes in mouse-human hybrid clone, 70-62, following BrdU-AO staining. Human chromosomes 11 and 17 (*arrow*) and del(X) (*bolder arrow*) are present in this metaphase. Late replication of the del(X) is *indicated by its faint staining*.

This, however, may not be the case when the Xg antigen gene is on the X chromosome that is preferentially inactivated in every cell. In any event, our results utilizing physical separation of active and inactive X chromosomes by segregation in somatic-cell hybrids clearly indicate that the distal short arm of the X can escape inactivation on both structurally normal and aberrant human X chromosomes.

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REFERENCES

1. SHAPIRO LJ, WEISS R, WEBSTER D, FRANCE JT: X-linked ichthyosis due to steroid sulfatase deficiency. *Lancet* i:70–72, 1978
2. MOHANDAS T, SHAPIRO LJ, SPARKES RS, SPARKES MC: Regional assignment of the steroid sulfatase-X-linked ichthyosis locus: implications for a non-inactivated region on the short arm of human X chromosome. *Proc Natl Acad Sci USA* 76:5779–5783, 1979
3. TIEPOLO L, ZUFFARDI O, FRACCARO M, ET AL.: Assignment by deletion mapping of the steroid sulfatase X-linked ichthyosis locus to Xp223. *Hum Genet* 54:205–206, 1980

4. FERGUSON-SMITH MA, SANGER R, TIPPETT P, AITKEN DA, BOYD E: A familial t(X;Y) translocation which assigns the Xg blood group locus to the region Xp22.3→pter. *Cytogenet Cell Genet* 32:273–274, 1982
5. CURRY CJR, LANMAN JT JR, MAGENIS RE, BROWN MG, BERGNER EA, SHAPIRO LJ: X-linked chondrodysplasia punctata with ichthyosis: chromosomal localization to Xp. *Am J Hum Genet* 34:122A, 1982
6. LYON MF: X-chromosome inactivation and developmental patterns in mammals. *Biol Rev* 47:1–35, 1972
7. SHAPIRO LJ, MOHANDAS T, WEISS R, ROMEO G: Non-inactivation of an X-chromosome locus in man. *Science* 204:1224–1226, 1979
8. MIGEON BR, SHAPIRO LJ, NORUM RA, MOHANDAS T, AXELMAN J, DABORA RL: Differential expression of steroid sulfatase locus on active and inactive human X chromosome. *Nature* 299:838–840, 1982
9. MOHANDAS T, SPARKES RS, HELLKUHL B, GRZESCHIK KH, SHAPIRO LJ: Expression of an X-linked gene from an inactive human X chromosome in mouse-human hybrid cells: further evidence for the noninactivation of the steroid sulfatase locus in man. *Proc Natl Acad Sci USA* 77:6759–6763, 1980
10. RACE RR, SANGER R: *Blood Groups in Man.* Oxford, England, Blackwell, 1975
11. POLANI PE, ANGELL R, GIANNELLI F, DE LA CHAPELLE A, RACE RR, SANGER R: Evidence that the Xg locus is inactivated in structurally abnormal X chromosomes. *Nature* 227:613–616, 1970
12. SANGER R, TIPPETT P, GAVIN J, TEESDALE P, DANIELS GL: Xg blood groups and sex chromosome abnormalities in people of northern European ancestry: an addendum. *J Med Genet* 14:210–213, 1977
13. PEARSON PL, WITTERLAND WF, MEERA-KHAN P, DEWITT J, BOBROW M: Reinvestigation of two X/autosome translocations: segregation in cell hybrids. *Cytogenet Cell Genet* 22:534–537, 1978
14. ROPERS HH, MIGL B, ZIMMER J, FRACCARO M, MARASCHIO PP, WESTERVELD A: Activity of steroid sulfatase in fibroblasts with numerical and structural X chromosome aberrations. *Hum Genet* 57:354–356, 1981
15. CHANCE PF, GARTLER SM: Evidence for a dosage effect at the X-linked steroid sulfatase locus in human tissues. *Am J Hum Genet* 35:234–240, 1983
16. MULLER CR, MIGL B, TRAUPE H, ROPERS HH: X-linked steroid sulfatase: evidence for different gene dosage in males and females. *Hum Genet* 54:197–199, 1980
17. EPSTEIN EH, LEVENTHAL ME: Steroid sulfatase of human leukocytes and epidermis and the diagnosis of recessive X-linked ichthyosis. *J Clin Invest* 67:1257–1262, 1981
18. MILLER OJ, SINISCALCO M: Report of the Committee on the Genetic Constitution of the X and Y Chromosomes. *Cytogenet Cell Genet* 32:179–190, 1982
19. MOHANDAS T, SPARKES RS, SHAPIRO LJ: Reactivation of the inactive human X chromosome: evidence for X-inactivation by DNA methylation. *Science* 211:393–396, 1981
20. PAI GS, SPRINKLE JT, DO TT, MARENI CE, MIGEON BR: Localization of loci for hypoxanthine phosphoribosyltransferase and glucose-6-phosphate dehydrogenase and biochemical evidence of nonrandom X chromosome expression from studies of a human X-autosome translocation. *Proc Natl Acad Sci USA* 77:2810–2813, 1980
21. DAVIDSON RL, GERALD PS: Improved techniques for the induction of mammalian cell hybridization by polyethylene glycol. *Somat Cell Genet* 2:165–176, 1976
22. NORWOOD TH, ZEIGLER CJ, MARTIN GM: Dimethylsulfoxide enhances polyethylene glycol-mediated somatic cell fusion. *Somat Cell Genet* 2:263–270, 1976
23. SZYBALSKA EH, SZYBALSKI W: Genetics of human cell lines IV. DNA-mediated heritable transformation of a biochemical trait. *Proc Natl Acad Sci USA* 48:2026–2034, 1962
24. LITTLEFIELD JW: Selection of hybrids from matings of fibroblasts in vitro and their presumed recombinants. *Science* 145:709–710, 1964
25. KUCHERLAPATI RS, BAKER RM, RUDDLE FH: Ouabain as a selective agent in the isolation of somatic cell hybrids. *Cytogenet Cell Genet* 14:326–363, 1975

26. SHAPIRO LJ, WEISS R, BUXMAN MM, ET AL.: Enzymatic basis of typical X-linked ichthyosis. *Lancet* ii:756–757, 1978
27. SPARKES RS, BALUDA MC, TOWNSEND DE: Cellulose acetate electrophoresis of human glucose-6-phosphate dehydrogenase. *J Lab Clin Med* 73:531–534, 1969
28. BEUTLER E: Electrophoresis of phosphoglycerate kinase. *Biochem Genet* 3:189–195, 1969
29. DUTRILLAUX B: Studies of human X-chromosomes with the 5-BudR-acridine orange technique: application to X-chromosome pathology, in *Chromosomes Today*, vol 5, edited by PEARSON PL, LEWIS KR, New York, John Wiley, 1976, pp 395–407
30. GOODFELLOW P, PYM B, MOHANDAS T, SHAPIRO LJ: The *MIC2X* locus escapes X-inactivation. *Am J Hum Genet* 36:777–782, 1984
31. GOODFELLOW P, BANTING G, SHEER D, ET AL.: Genetic evidence that a Y-linked gene in man is homologous to a gene on the X-chromosome. *Nature* 302:346–349, 1983

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