Am. J. Hum. Genet. 45:592–598, 1989

Noninactivation of a Selectable Human X-linked Gene That Complements a Murine Temperature-sensitive Cell Cycle Defect

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Summary

The human gene *A1S9T,* which complements the temperature-sensitive cell-cycle defect in the murine cell line tsA1S9 and which has previously been assigned to the X-chromosome short arm, is expressed from the inactive X chromosome in human/tsA1S9 somatic cell hybrids grown at the nonpermissive temperature. The Y chromosome cannot complement the defect; thus, unlike at least two other noninactivated X loci, *A1S9T* has no functional Y-linked homologue. As *A1S9T* is readily selectable in somatic cell hybrids with the tsA1S9 mouse line, this marker should be useful in isolating somatic cell hybrids containing inactive X chromosomes, or abnormal X's (active or inactive) retaining the short arm.

Introduction

Genes located on the X chromosome in eutherian females are subject to a unique form of developmental gene regulation, known as X-chromosome inactivation (Lyon 1972, 1988; Gartler and Riggs 1983). This inactivation event is a cis-acting regulatory process affecting nearly the entire chromosome—in the case of the human X chromosome about 160 megabase pairs of DNA. Previously four genes have been shown to escape this chromosome-wide inactivation. These are the genes for the MIC2 cell surface antigen (*MIC2*) (Goodfellow et al. 1984), the microsomal enzyme steroid sulfatase (*STS*) (Shapiro et al. 1979), the Xg red cell antigen (*XG*) (Race and Sanger 1975), and *ZFX,* an X-linked homologue of the putative sex-determining gene *ZFY* (Schneider-Gadicke et al., in press). Three of these genes are clustered in the distal short arm of the X chromosome, in or near the "pseudoautosomal" region which is shared by the X and Y chromosomes and known to undergo recombination during male meiosis (Burgoyne 1982). The fourth, *ZFX,* is located more proximally in Xp21.3-Xp22.1 (Page et al. 1987; Schneider-Gadicke et al., in press).

The mechanism of X inactivation is not known, although it has been shown that the inactive X chromosome (Xi) is heteropyknotic, late-replicating, and methylated differently relative to the active X chromosome (Gartler and Andina 1976; Gartler and Riggs 1983). These characteristics do not seem to apply to the genes which escape X inactivation, as the pseudoautosomal region has been observed to be early replicating (Schempp and Meer 1983), and the *MIC2* gene does not show the methylation differences seen with X-linked housekeeping genes which are inactivated (Goodfellow et al. 1988).

Elsewhere we have described a gene located on the short arm of the X chromosome which complements the temperature-sensitive defect of the mutant murine cell line tsA1S9 (Brown et al. 1989). At the nonpermissive temperature (npt) of 39°C, tsA1S9 cells are unable to synthesize high-molecular-weight DNA from newly replicated single-stranded DNA, and thus they fail to survive (Sheinin 1976). We created somatic cell hybrids by fusing the mutant murine line with human fibroblasts at the permissive temperature. Only hybrids containing the human X chromosome short arm were able to grow at the npt, and thus the *A1S9T* gene could be assigned to the X short arm (Brown et al. 1989).

In the present study we have determined that this gene escapes the process of X-chromosome inactivation. We

Received April 14, 1989; revision received June 12, 1989.

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0002-9297/89/4504-0013$02.00

also show that the gene has no functional Y-linked homologue and thus must not be pseudoautosomal. The *A1S9T* gene is readily selectable in mouse/human hybrid cell lines, allowing for both the selection of the short arm of the X chromosome and inactive X chromosomes which should be important both for X-chromosome mapping and studying the inactivation process.

Material and Methods

Murine/Human Somatic Cell Hybrids

Human skin fibroblasts were grown at 37°C in alpha-MEM supplemented with 15% FBS. tsA1S9az31B, a hypoxanthine guanine phosphoribosyltransferase-deficient ($HPRT^-$) derivative of the mutant murine line (Brown et al. 1989), was maintained at 34°C in alpha-MEM supplemented with 7.5% FBS. Somatic cell hybrids were created by fusing tsA1S9az31B with various human fibroblasts. Fusions and subsequent chromosome analysis were as described elsewhere (Brown et al. 1989). Selection for fusants used HAT medium (10^{-4} M hypoxanthine, 1.6×10^{-5} thymidine, 4.2×10^{-4} aminopterin) or the npt to select against the murine parental line and ouabain to select against the human parental line. For direct selection of hybrids containing an inactive X chromosome, growth at the npt selected against the tsA1S9 parental line and growth in azaguanine and thioguanine (AG/TG; 2×10^{-4} M 8-azaguanine, 6×10^{-5} M 6-thioguanine) selected against both the human parental lines and hybrids containing an active X chromosome. The human parental lines for each hybrid series are outlined in table 1. Back-selection against the active human X chromosome was accomplished by selection against HPRT expression from the long arm of the chromosome by growth in media containing AG/TG.

Assays for X-Chromosome Activity

Replication analysis of the X chromosome was performed after growth in medium supplemented with 5-bromodeoxyuridine (BrdUrd) (Willard and Latt 1976). Hoescht 33258-Giemsa staining for replication bands was performed according to a method described elsewhere (Willard 1977). HPRT activity was analyzed by growth in selective media. As the parental mouse line was $HPRT^-$, all HPRT activity was presumed to be due to the human gene. Hybrids with HPRT activity were able to grow in HAT medium and not in AG/TG medium, while hybrids which were not expressing HPRT could survive in AG/TG medium but not in HAT medium. Expression of the human glucose-6-phosphate dehydrogenase (*G6PD*) gene was determined by electrophoresis of whole-cell extracts (Meera Khan 1971). To analyze methylation at the 5′ end of the phosphoglycerate kinase gene (*PGK1*), genomic DNA (10 μg) was digested either with *Sau*3A or with *Bam*HI and *Eco*RI, alone or with *Hpa*II, according to a method described by Keith et al. (1986). Southern blots of this DNA were hybridized with an 800-bp *Bam*HI/*Eco*RI fragment from the 5′ end of the *PGK1* gene. The conditions for Southern transfer and hybridization have been described elsewhere (Willard et al. 1983). Steroid sulfatase activity in whole-cell sonicates was assayed according to a method described elsewhere (Willard and Holmes 1984).

Table 1

Chromosome Complement of Human Fibroblasts Used in Fusions

Hybrid Series	Karyotype	Derivative X Chromosomes	Reference[a]
t60	46,XX	No detectable cytological alteration	
t11	46,XX	No detectable cytological alteration	
t48	46,X,t(X;11) (p11.1;p13) [GM2859]	11q/Xq (11qter→11p13::Xp11.1→Xqter) Xp/11p (Xpter→Xp11.1::11p13→11pter)[b]	1
t81	46,X,del(X)	Xq-(Xpter→Xq13:)[c]	2
t86	46,X,t(X;11) (p2105;q13) [GM1695]	11q/Xp (11pter→11q13::Xp2105→Xpter) Xq/11p (Xqter→Xp2105::11q13→11qter)[b]	1
t4	47,Y,t(X;14) (q13;q32), + der(14) [GM0074]	Xp/14q (Xpter→Xq13::14q32→14qter) 14p/Xq (14pter→14q32::Xq13→Xqter) (Two copies of 14p/Xq)[d]	1

[a] 1 = NIGMS Human Genetic Mutant Cell Repository (1986); 2 = Tantravahi et al. (1983).
[b] In the balanced X/autosome translocations the normal X chromosome is inactive.
[c] The deleted X chromosome is inactive in line 81.
[d] One of the 14p/Xq chromosomes is inactivated in cell line 4.

Results

A1S9T Is Expressed from the Inactive X Chromosome

We have demonstrated elsewhere that tsA1S9-human somatic cell hybrids which contain an X chromosome are able to survive at the npt, while back-selection against the X results in hybrids without the X that are unable to survive at the npt (Brown et al. 1989). However, we isolated one hybrid, t11–4Aaz5, which continued to survive at the npt in AG/TG media. This hybrid continued to express human STS which is expressed from both active and inactive X chromosomes, but it did not express the human X-linked enzyme G6PD, which is known to be subject to X inactivation (Davidson et al. 1963) (table 2). Cytogenetic analysis of t11–4Aaz5 showed that it contained an inactive X chromosome, as judged by replication analysis after growth in BrdUrd (Willard and Latt 1976) (data not shown).

Four independent sets of tsA1S9-human somatic cell hybrids have yielded hybrids which contain an inactive X (Xi) and not an active X (Xa) and which are able to grow at the npt (table 2). There is no other human chromosome in common in these hybrids. In contrast, we have never isolated a temperature-sensitive hybrid with an inactive X chromosome. Even at the permissive temperature, we have observed a high frequency of hybrids maintaining the Xi, and at the npt many of the Xi hybrid cell lines contain more than one Xi per cell. One hybrid was observed to have as many as seven late-replicating Xi's per cell (see fig. 1).

Table 2 outlines both the X-chromosomal material present in these cell lines and the evidence for the Xi being inactive. The X-linked genes for the enzymes HPRT, G6PD, and PGK are known to be subject to X-chromosome inactivation (Davidson et al. 1963; Migeon et al. 1968; Gartler et al. 1972). We assessed the activity of these enzymes by three different techniques. To monitor the activity state of the X-linked human *PGK1* gene, we analyzed methylation at the 5′ region of the gene, which is known to reflect activity of the X chromosome (Keith et al. 1986) (fig. 2*A*). Methylation was assessed by using the methylation-sensitive enzyme *Hpa*II, which can only digest DNA if the internal C of its CCGG recognition site is unmethylated.

Table 2

Characteristics of Inactive X-Chromosome–containing Somatic Cell Hybrids

Hybrid Cell Line[a]	X-Chromosomal Material[b]		Growth at 39°C[c]	Methylation at PGK1[d]	HPRT Activity[e]	G6PD Activity[f]
	Xa	Xi				
t60-12	+	–	+	A	+	+
t11-4A	+	+	+	A/I	+	+
t11-4Aaz5	–	+	+	I	–	–
t48-1a-1D	+(11q/Xq)	+	+	A/I	+	+
t48-1a-2a	+(11q/Xq)	–	–	A	+	+
t48-1a-1Daz4A	–	+	+	I	–	–
t81	+	+(Xq-)	+	A/I	+	+
t81-az1D	–	+(Xq-)	+	I	–	–
t86-Blm	+(11q/Xp)	+	+	A/I	+	+
t86-Blmaz1b	–	+	+	I	–	–

[a] Hybrid cell lines were derived from somatic cell fusion of the tsA1S9 murine line with different human parental lines, as described in Material and Methods and outlined in table 1.

[b] Chromosomal material present in each hybrid was determined by analysis of trypsin-Giemsa karyotypes of metaphase chromosomes. Presence of a chromosome is indicated by a plus sign (+), followed by a description of the chromosome if it is not cytologically normal. The derivative X chromosomes were as described in table 1. Xa = active X chromosomes; Xi = inactive X chromosomes. Activity status was determined by cytological examination for those hybrids in which the Xi has previously been demonstrated and is cytologically unique. For the t11 cell line, activity was determined by replication analysis.

[c] Ability of a cell line to grow was determined both by plating efficiency and by determining the ratio of tritiated thymidine incorporated at the npt vs. the pt (Brown et al. 1989).

[d] Assay for methylation in the 5′ region of the *PGK1* gene is shown in fig. 2A. I = methylated/inactive X chromosome; A = unmethylated/active X chromosome; A/I = intermediate pattern (Keith et al. 1986).

[e] Determined by the ability of the hybrid to grow in selective media. A plus sign (+) indicates resistance to HAT medium and sensitivity to AG/TG medium, while a minus sign (–) indicates HAT sensitivity and AG/TG resistance.

[f] Determined by the presence of the human-specific isozyme on protein gel electrophoresis (see fig. 2*B*).

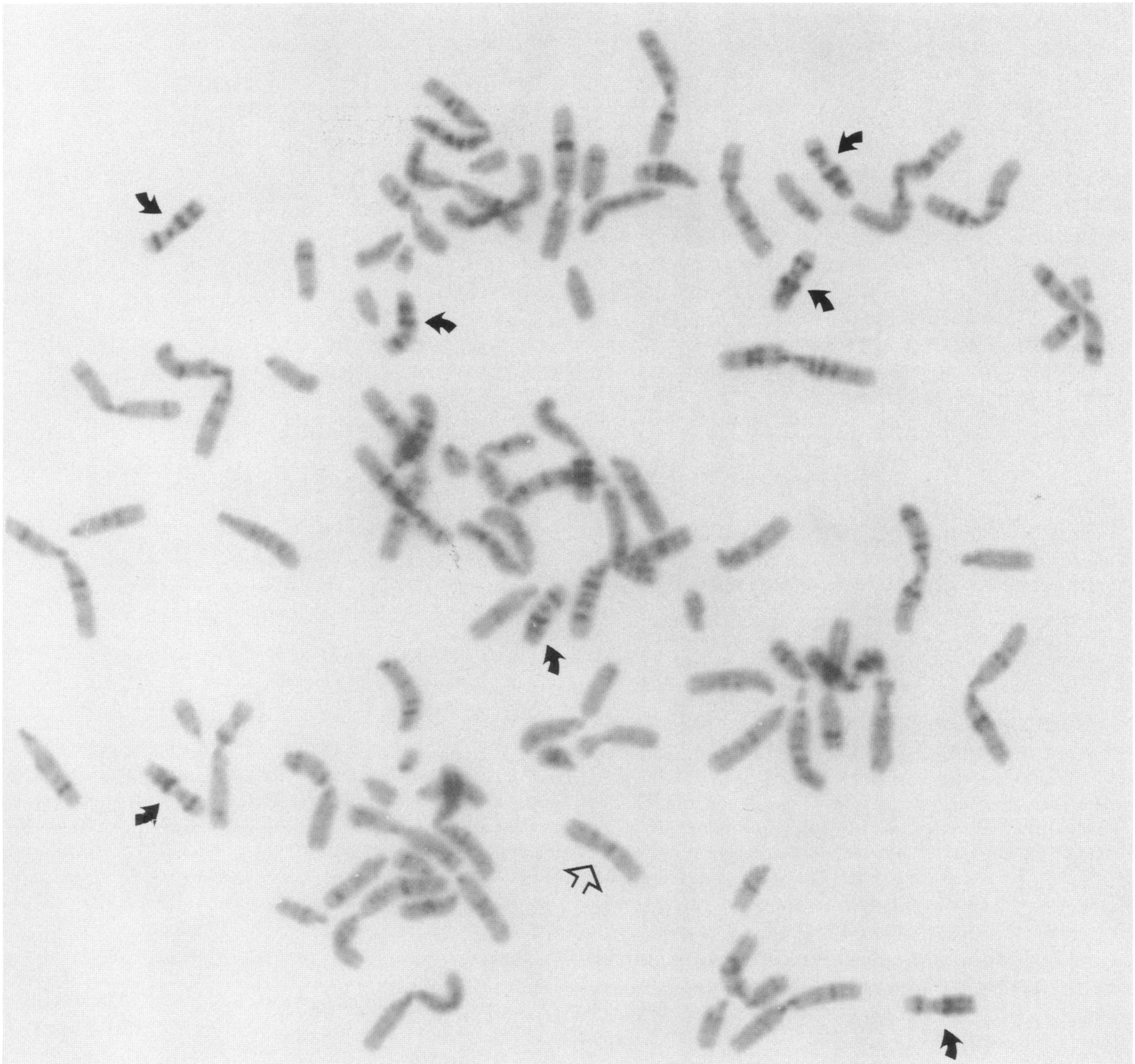

Figure 1 Micrograph of a Hoechst 33258-Giemsa–stained metaphase spread of chromosomes from a subclone of the t48 hybrid after growth in BrdUrd. The normal Xi's are indicated by solid arrows, and the translocated Xq/11q is indicated by the open arrow. Cells were grown at 39°C before chromosome analysis.

Restriction-enzyme digests without *Hpa*II yield a fragment of 807 bp (for *Bam*HI/*Eco*RI digests) or 662 bp (for *Sau*3A digests). These fragments from the 5′ end of the *PGK1* gene contain multiple *Hpa*II sites, so that, when *Hpa*II is included in the digests, they are digested into many smaller, unresolved bands. However, if *Hpa*II is unable to cut because the *Hpa*II sites are methylated (as they are on inactive chromosomes), then digestion at the multiple *Hpa*II sites is prevented and the full-size fragment is detected (Keith et al. 1986). On the basis of the results of this assay, this region was methylated (and therefore inactive) in all four Xi hybrids examined (fig. 2*A*). Consistent with this result, human G6PD activity could not be demonstrated in any of the Xi hybrids (fig. 2*B*). On the basis of (1) these data, (2) failure of any of the Xi hybrids to survive in HAT medium, and

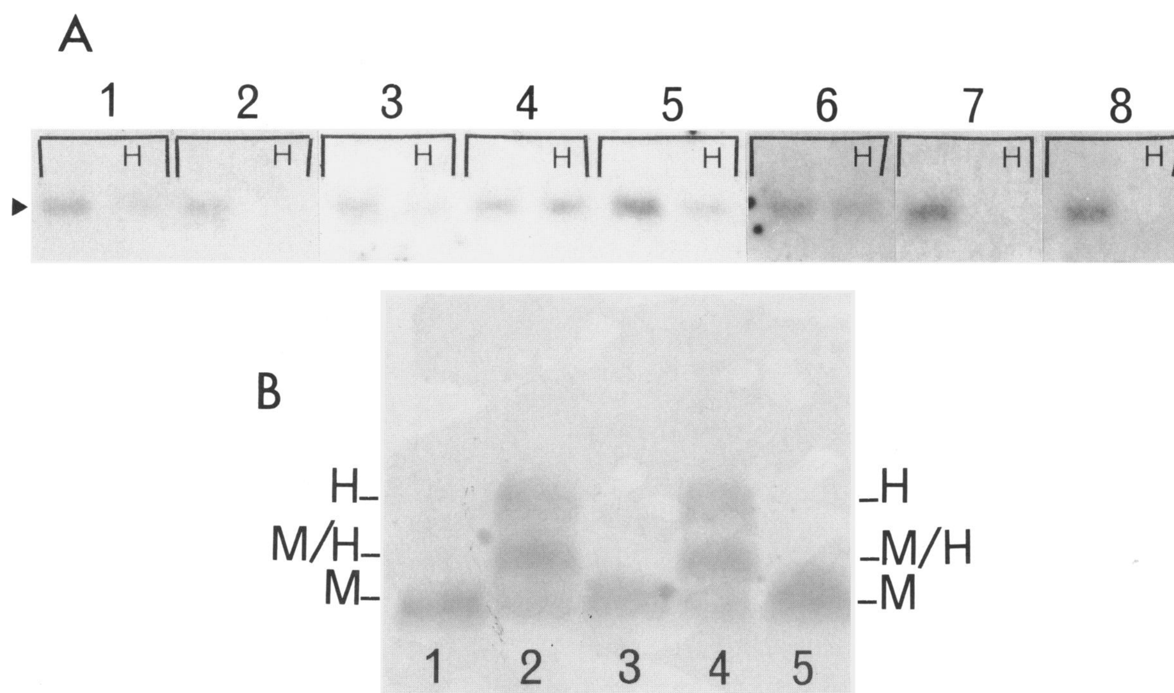

Figure 2 *A*, Methylation at the 5′ end of the *PGK1* gene has been correlated with activity of the X chromosome (Keith et al. 1986). Using an 800-bp *Bam*HI/*Eco*RI fragment as a probe for Southern blotting, we assessed methylation by cutting with *Bam*HI and *Eco*RI (lanes 1–5) or *Sau*3A (lanes 6–8) with (lanes marked "H") or without *Hpa*II. Methylation of the dinucleotide CpG inhibits cutting of the *Hpa*II enzyme, resulting in release of the intact fragment (807 bp for lanes 1–5 and 662 bp for lanes 6–8; marked by triangle). However, if the *Hpa*II sites are unmethylated, as they are on active chromosomes, then digestion at the multiple *Hpa*II sites within this region occurs, and no band is detected. The DNA samples are as follows: 1 = female; 2 = male; 3 = t48-1a-1D; 4 = t48-1a-1Daz4A; 5 = t11-4A; 6 = t11-4Aaz5; 7 = male; 8 = t60-12. *B*, Expression of the X-linked enzyme G6PD. Cellogel electrophoresis separates the human (H) and mouse (M) isozymes, as well as a human/mouse heterodimer (H/M), all of which are detected by histochemical staining (Meera Khan 1971). Sources of the cell extracts were as follows: 1 = tsA1S9 mouse parental line; 2 = t81; 3 = t81-az1D; 4 = t86B1m; 5 = t86-B1maz1b.

(3) late replication of the X chromosomes maintained in these hybrids, we conclude that the X's are inactive (table 2).

A1S9T Is Not Pseudoautosomal

To determine whether the Y chromosome can complement the tsA1S9 defect, we created somatic cell hybrids between tsA1S9 and a male cell line, GM074 (hybrid series t4; table 1). Of 13 hybrids able to survive the npt (selected by virtue of the *A1S9T* locus), all retained the X-chromosome short arm (as part of a X;14 translocation; see table 1), but only three retained the Y chromosome. One additional hybrid, isolated at the permissive temperature, retained a Y chromosome but no X-chromosome short arm. This hybrid was unable to grow at the npt, indicating that the Y chromosome does not contain a functional *A1S9T* gene.

Discussion

Three of the genes previously reported to be expressed from the Xi are located near the distal tip of the X-chromosome short arm. One of these genes, *MIC2*, is pseudoautosomal and is expressed from both the X and Y chromosomes. We have reported elsewhere that the *A1S9T* gene maps to the short arm of the X chromosome (Brown et al. 1989); however, since our fusions were performed with female cell lines, we had not addressed whether the Y chromosome could also complement the tsA1S9 defect. In the present study, we have created fusions with a male cell line and have observed no evidence that the Y chromosome is capable of complementing the defect. Thus, the *A1S9T* locus is not expressed from the Y chromosome and must, therefore, be located below the pseudoautosomal boundary in Xp22.3 (Ellis et al. 1989). It is unlikely that *A1S9T*

is distal to *XG* and *STS,* since males with deletions or unbalanced translocations resulting in nullisomy for the distal portion of the X are asymptomatic except for ichthyosis from the loss of *STS* (Geller et al. 1986). Thus, *A1S9T* must be more proximal than these other noninactivated X-linked genes. Studies are in progress to further localize the complementing activity by fusion of tsA1S9 with human cell lines carrying breakpoints in the short arm of the X chromosome, proximal to *STS.*

While it is generally assumed that most of the estimated several thousand X-linked genes are subject to X inactivation, there is only firm evidence that approximately 35 genes are inactivated, less than 10-fold more than the number known to escape inactivation. This estimate is based primarily either on the number of genes showing clinical expression of disease phenotypes in heterozygotes with X/autosome translocations (reviewed in Harper et al. 1988) or by demonstrated clonality in cultured cells from heterozygotes (e.g., see Salzmann et al. 1968; Meyer et al. 1975; Migeon et al. 1981). There is also evidence from patchy expression in heterozygous females (Passerge and Fries 1973), from expression studies in somatic cell hybrids (e.g., see Graves and Gartler 1986; Nadon et al. 1988), and from the demonstration of allelic exclusion (e.g., see Gealy et al. 1980; Fearon et al. 1987). In addition to the X-linked genes for which direct evidence of X inactivation exists, there are a considerably larger number of X-linked clinical defects that show limited expression in females, a finding that may suggest that they are also subject to X inactivation (McKusick 1988). Nonetheless, it seems reasonable to expect that, as the number of genes identified on the X increases, more genes will be found to escape X-chromosome inactivation. The identification of these genes may be of practical importance for individuals who have only one X chromosome (Turner syndrome) or multiple X chromosomes, since females with only one X chromosome will have expression similar to males rather than to females, while individuals with multiple X chromosomes will have overexpression of these genes. The molecular characterization of *A1S9T* and other genes which escape X inactivation could help determine the mechanism by which genes escape inactivation and therefore, perhaps, how the process of inactivation itself effects gene expression on the X.

A1S9T is unique among the genes known to escape X inactivation, because it allows for the direct selection of inactive X chromosomes in tsA1S9-human somatic cell hybrids. The observation that there is often more than one Xi retained per cell at the npt demonstrates the strength of the selective pressure to retain the Xi in tsA1S9-human somatic cell hybrids, and it may suggest an underexpression of the gene from the Xi, as has been shown for some X-linked genes (Migeon et al. 1982*a,* 1982*b*) but apparently not for others (Schneider-Gadicke et al., in press). The isolation of Xi hybrids should be useful for furthering the study of many aspects of the process of X-chromosome inactivation.

In addition, *A1S9T* provides a second selectable marker for the X chromosome, this one on the short arm of the chromosome, which should enhance mapping efforts on the X. With this system, one can select for structurally abnormal inactive X chromosomes, as demonstrated here by one of the Xi hybrids isolated containing a deleted Xi. These hybrids should prove useful in localizing regions necessary for X-chromosome inactivation, including the putative X inactivation center(s).

Acknowledgments

We thank V. E. Powers for assistance with chromosome analysis and Drs. P. Goodfellow and D. Page for helpful discussions. This study was supported by a grant from the Medical Research Council of Canada. H.F.W. was the recipient of a Scientist Award from the Medical Research Council of Canada.

References

Brown CJ, Powers VE, Monroe DM, Sheinin R, Willard HF (1989) A gene on the short arm of the human X chromosome complements the murine tsA1S9 DNA synthesis mutation. Somatic Cell Mol Genet 15:173–178

Burgoyne PS (1982) Genetic homology and crossing over in the X and Y chromosomes of mammals. Hum Genet 61:85–90

Davidson RG, Nitowsky HM, Childs B (1963) Demonstration of two populations of cells in the human female heterozygous for glucose-6-phosphate dehydrogenase variants. Proc Natl Acad Sci USA 50:481–485

Ellis NA, Goodfellow PJ, Pym B, Smith M, Palmer M, Frischauf A-M, Goodfellow PN (1989) The pseudoautosomal boundary in man is defined by an Alu repeat sequence inserted on the Y chromosome. Nature 337:81–84

Fearon ER, Winkelstein JA, Civin CI, Pardoll DM, Vogelstein B (1987) Carrier detection in X-linked agammaglobulinemia by analysis of X-chromosome inactivation. N Engl J Med 316:427–431

Gartler SM, Andina RJ (1976) Mammalian X-chromosome inactivation. In: Harris H, Hirschhorn K (eds) Advances in human genetics. Vol 7. Plenum, New York, pp 99–140

Gartler SM, Chen S-H, Fialkow PJ, Giblett ER (1972) X chromosome inactivation in cells from an individual heterozygous for two X-linked genes. Nature New Biol 236:149–150

Gartler SM, Riggs AD (1983) Mammalian X-chromosome inactivation. Annu Rev Genet 17:155–190

Gealy WJ, Dwyer JM, Harley JB (1980) Allelic exclusion of glucose-6-phosphate dehydrogenase in platelets and T lymphocytes from a Wiskott-Aldrich syndrome carrier. Lancet 1:63–65

Geller RL, Shapiro LJ, Mohandas TK (1986) Fine mapping of the distal short arm of the human X chromosome using X/Y translocations. Am J Hum Genet 38:884–890

Goodfellow P, Pym B, Mohandas T, Shapiro LJ (1984) The cell surface antigen locus, MIC2X, escapes X-inactivation. Am J Hum Genet 36:777–782

Goodfellow PJ, Mondello C, Darling SM, Pym B, Little P, Goodfellow PN (1988) Absence of methylation of a CpG-rich region at the 5′ end of the MIC2 gene on the active X, the inactive X, and the Y chromosome. Proc Natl Acad Sci USA 85:5605–5609

Graves JAM, Gartler SM (1986) Mammalian X chromosome inactivation: testing the hypothesis of transcriptional control. Somatic Cell Mol Genet 12:275–280

Harper P, Frezal J, Ferguson-Smith M, Shinzel A (1988) Report of the Committee on Clinical Disorders and Chromosomal Deletion Syndromes. Cytogenet Cell Genet 49: 254–256

Keith DH, Singer-Sam J, Riggs AD (1986) Active X chromosome DNA is unmethylated at eight CCGG sites clustered in a guanine-plus-cytosine-rich island at the 5′ end of the gene for phosphoglycerate kinase. Mol Cell Biol 6:4122–4125

Lyon MF (1972) X-chromosome inactivation and developmental patterns in mammals. Biol Rev 47:1–35

——— (1988) X-chromosome inactivation and the location and expression of X-linked genes. Am J Hum Genet 42:8–16

McKusick VA (1988) Mendelian inheritance in man. 8th ed. Johns Hopkins University Press, Baltimore

Meera Khan P (1971) Enzyme electrophoresis on cellulose acetate gel: zymogram patterns in man-mouse and man-chinese hamster somatic cell hybrids. Arch Biochem Biophys 145:470–483

Meyer WJ, Migeon BR, Migeon CJ (1975) Locus on human X chromosome for dihydrotestosterone receptor and androgen insensitivity. Proc Natl Acad Sci USA 72:1469–1472

Migeon BR, der Kaloustian VM, Nyhan WL, Young WJ, Childs B (1968) X-linked hypoxanthine-guanine phosphoribosyl transferase deficiency: heterozygote has two clonal populations. Science 160:425–427

Migeon BR, Moser HW, Moser AB, Axelman J, Sillence D, Norum RA (1981) Adrenoleukodystrophy: evidence for X linkage, inactivation, and selection favoring the mutant allele in heterozygous cells. Proc Natl Acad Sci USA 78: 5066–5070

Migeon BR, Shapiro LJ, Norum RA, Mohandas T, Axelman J, Dabora RL (1982*a*) Differential expression of steroid sulphatase locus on active and inactive human X chromosome. Nature 299:838–840

Migeon BR, Wolf SF, Mareni C, Axelman J (1982*b*) Derepression with decreased expression of the G6PD locus on the inactive X chromosome in normal human cells. Cell 29:595–600

Nadon N, Korn N, DeMars R (1988) A-11: cell type-specific and single-active-X transcription controls of newly found gene in cultured human cells. Somatic Cell Mol Genet 14:541–552

NIGMS Human Genetic Mutant Cell Repository (1986) Catalog of Cell Lines. National Institutes of Health, Bethesda, MD

Page DC, Mosher R, Simpson EM, Fisher EMC, Mardon G, Pollack J, McGillivray B, et al (1987) The sex-determining region of the human Y chromosome encodes a finger protein. Cell 51:1091–1104

Passerge E, Fries E (1973) X chromosome inactivation in X-linked hypohidrotic ectodermal dysplasia. Nature New Biol 245:58–59

Race RR, Sanger R (1975) The Xg blood groups In: Blood groups in man. Blackwell, Oxford, pp 579–593

Salzmann J, DeMars R, Benke P (1968) Single-allele expression at an X-linked hyperuricemia locus in heterozygous human cells. Proc Natl Acad Sci USA 60:545–552

Schempp W, Meer B (1983) Cytologic evidence for three human X-chromosomal segments escaping inactivation. Hum Genet 63:171–174

Schneider-Gadicke A, Beer-Romero P, Brown LG, Nussbaum R, Page DC. The ZFX gene on the human X chromosome escapes X inactivation and is closely related to ZFY, the putative sex determinant on the Y chromosome. Cell (in press)

Shapiro LJ, Mohandas T, Weiss R, Romeo G (1979) Non-inactivation of an X-chromosome locus in man. Science 204:1224–1226

Sheinin R (1976) Preliminary characterization of the temperature-sensitive defect in DNA replication in a mutant mouse L cell. Cell 7:49–57

Tantravahi U, Kirshner DA, Beauregard L, Page L, Kunkel L, Latt S (1983) Cytologic and molecular analysis of 46XXq-cells to identify a DNA segment that might serve as a probe for a putative human X chromosome inactivation center. Hum Genet 64:33–38

Willard HF (1977) Tissue-specific heterogeneity in DNA replication patterns of human X chromosomes. Chromosoma 61:61–73

Willard HF, Holmes MT (1984) A sensitive and dependable assay for distinguishing hamster and human X-linked steroid sulfatase activity in somatic cell hybrids. Hum Genet 66:272–275

Willard HF, Latt SA (1976) Analysis of deoxyribonucleic acid replication in human X chromosomes by fluorescence microscopy. Am J Hum Genet 28:213–227

Willard HF, Smith KD, Sutherland J (1983) Isolation and characterization of a major tandem repeat family from the human X chromosome. Nucleic Acids Res 11:2017–2033