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Dosage of the *Sts* Gene in the Mouse

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SUMMARY

In this study we compared steroid sulfatase levels in XO, XX, and XY mice and carried out a clonal analysis in fibroblast cell cultures from mice heterozygous for the steroid sulfatase deficiency gene and heterozygous at the X-linked electrophoretic phosphoglycerate kinase locus. The combined results indicate that the murine steroid sulfatase locus is not dosage compensated and is not subject to X-inactivation. With respect to X-inactivation, it behaves in a somewhat different way from the closely linked sex-reversed gene and the human steroid sulfatase locus.

INTRODUCTION

We have recently presented evidence for the existence of functional Y- and X-linked alleles for the steroid sulfatase (*Sts*) locus in the mouse [1]. The locus appears to be located in the XY pairing region with the alleles undergoing obligatory recombination during male meiosis. Since there are two copies of the *Sts* gene in both male and female mice, there would appear to be no need for dosage compensation at this locus. However, previous reports comparing STS levels in XO, XX, and XY somatic tissues have been conflicting with respect to the presence or absence of dosage-compensating effects [2, 3]. Furthermore, the mouse sex-reversed gene (*Sxr*), which must be closely linked to *Sts* [4, 5], can be subject to X-inactivation [6, 7], and the human *Sts* gene is subject to partial X-inactivation [8–11]. Thus, there is considerable justification for an investigation of dosage compensation at this locus in the mouse. Here, we compared STS levels in XO, XX, and XY mice and carried out a clonal analysis in fibroblast cell cultures from mice heterozygous for the *Sts* deficiency gene

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and heterozygous at the X-linked electrophoretic phosphoglycerate kinase (*Pgk*) locus. The combined results indicate that the murine *Sts* locus is not dosage compensated and is not subject to X-inactivation. With respect to X-inactivation, it behaves in a somewhat different way from the closely linked *Sxr* gene and the human *Sts* locus.

MATERIALS AND METHODS

Dosage Studies

An STS-positive tabby XO mouse (F_1 mice from C57BL/6J × CBA/Ca) was mated with an STS-positive BALB/c male. Tabby XY males, tabby heterozygous XX females, and XO mice with normal coat color were sacrificed and samples were taken from an ear punch, the kidney, and the spleen for STS assays and protein determinations. Ear punch samples for XX:XY ratio were obtained from another STS-positive mating. All STS assays were done in duplicate.

Clonal Studies

A C3H/An STS^- female that carried the *Pgk* B [12] allele was mated to a BALB/c STS^+ male carrying the *Pgk* A allele. Fibroblast cultures were established from ear punch biopsy samples of the F_1 STS^+ *Pgk* AB female. This doubly heterozygous culture was cloned (10% plating efficiency), colonies picked at 14 days, transferred to new flasks, and transformed with SV40. SV40 transformation was necessary to obtain sufficient numbers of clones. Evidence from previous studies suggest it does not affect X-inactivation [13]. When clones reached confluency, they were trypsinized, pelleted, washed one time with phosphate-buffered saline (PBS), and stored at −60°C until they were assayed for STS activity and electrophoretic mobility of PGK.

STS Assays

Kidney and spleen samples were homogenized and diluted to the same concentration (0.5 ml, 0.1 M Tris-HCl, pH 7.2). The homogenate was centrifuged (30 seconds, 12,000 *g*), and the supernatant was then frozen and thawed three times. Ear punch samples were treated as previously described [1]. Cell pellets from clones were diluted (250 μl, 0.1 M Tris-HCl, pH 7.2), sonicated on ice for 3 seconds at 100 W, and then frozen and thawed one time. The STS assay was performed on 50 μl of extract as reported using [^{3}H]estrone sulfate as the substrate [1]. Protein assays were done according to the Lowry method, and results are reported as pmol/hr per mg protein [14].

PGK Electrophoresis

Samples of each clone were run on cellulose acetate strips and stained for PGK activity according to a modification (L. A. Reddy, personal communication) of Meera Khan et al. [15]. The mobility was compared to PGK B controls on the same strip and classified as PGK A or PGK B.

RESULTS

Dosage Studies

STS activity levels for all tissues measured from XX and XO siblings of STS^+ × STS^+ matings indicate a simple dosage effect at the STS locus. All showed an approximate ratio of 2:1 (table 1). The means of enzyme activity in XX vs. XO animals were significantly different (kidney, $P < .05$; spleen, $P < .001$; ear, $P < .02$). On the other hand, XX and XY STS activity levels were not

TABLE 1

MEAN STS VALUES FROM INDIVIDUAL XX AND XO MICE IN THREE TISSUES (STS pmol/HR/mg PROTEIN)

	KIDNEY		SPLEEN		EAR PUNCH	
	XX	XO	XX	XO	XX	XO
	8.0	2.5	57.2	26.6	2030.0	588.9
	5.5	3.1	57.8	35.8	952.3	515.8
	9.6	4.6	61.8	19.2	1028.2	775.4
	6.5	6.1	43.3	34.4	1145.6	718.1
	9.0	3.4	39.7	21.3	907.4	728.3
	1.3	...	52.8	...	1094.0	...
	11.5	...	50.7	...	1085.7	...
	8.6	...	48.2	...	1128.0	...
$\bar{x}$	7.5	3.9	51.4	27.5	1171.4	665.3
(SD)	(3.1)	(1.4)	(7.5)	(7.5)	(356.8)	(108.5)
Ratio XX:XO	1.9		1.9		1.8	

NOTE: STS measured on eight XX and five XO mice in duplicate.

significantly different ($P > .4$) from each other (table 2). This supports previous data that suggest the presence of a functional Y-linked allele [1].

Clonal Studies

Clones were isolated from a fibroblast culture doubly heterozygous for STS expression variants and electrophoretic variants of the X-linked *Pgk* locus. The linkage arrangement in the culture was STS^- PGK B/STS^+ PGK A. STS activity was measured in duplicate for each clone and also for fibroblast cultures of the C3H/An STS^- parent and the BALB/c STS^+ parent. Two sets of clones were obtained with regard to PGK mobility (PGK A or B) due to random inactivation of the X chromosome (table 3). If X-inactivation inactivates the *Sts* gene, then clones that are PGK B should be STS^- and PGK A clones STS^+. If *Sts* is not inactivated, all the clones regardless of which X is inactivated should be STS^+. The results again confirm that *Sts* in the mouse is not inactivated. A *t*-test comparison of the means of two sets of the clones was not significantly different ($P > .4$).

TABLE 2

MEAN OF STS SAMPLES FROM XX AND XY MICE [STS pmol/HR/mg PROTEIN (SD)]

Sample	XX	XY	Ratio XX:XY
Ear punch*	344.0 (117.0)	399.9 (25.6)	0.9
Spleen†	51.4 (7.5)	43.2 (26.1)	1.2
Kidney†	7.5 (3.1)	9.0 (4.0)	0.8

* Mean STS values from 3XX, 3XY mice measured in duplicate.
† Mean STS values from 8XX, 5XY mice measured in duplicate.

TABLE 3

STS ACTIVITY FROM THE ACTIVE OR INACTIVE X (STS pmol/HR/mg PROTEIN)

P_1	C3H/An PGK B, STS$^-$	×	BALB/c PGK A, STS$^+$
	7.2		673.15
F_1	PGK AB, STS$^+$		
	PGK A (STS$^+$)		PGK B (STS$^-$)
Clones ..	307.8		899.6
	879.8		184.4
	398.0		821.1
	230.1		300.6
	840.6		168.7
	498.5		966.8
	1100.5		...
	822.0		...
	823.1		...
	1191.3		...
	691.1		...
	587.2		...
$\bar{x}$ (SD)	697.5 (301.9)		556.9 (376.9)

DISCUSSION

The results of our dosage compensation and X-inactivation studies of the murine *Sts* locus are compatible with the gene not being dosage compensated or subject to X-inactivation. If the *Sts* locus is subject to X-inactivation, the ratio of XX:XO STS activity should be 1:1. However, if both X-linked alleles remain active, then the ratio should be 2:1 as we found. These results differ from an earlier XX:XO dosage study in which a 1:1 ratio of STS activity was reported for kidney homogenates [2]. There are at least two factors that might have contributed to the difference between these experimental results. One involves inter- and even intra-strain variation in STS activity. We controlled for this variability in the present experiment by using successive litters from the same parental cross. Second, we realized in the present study that STS activity is linear with respect to protein concentration over a very narrow range [16], and we carefully diluted samples to the same protein concentration prior to assay.

In clonal studies, we measured STS activity in cells where we knew the *Sts*$^+$ allele was on the active or inactive X chromosome. The means of both sets of clones were not significantly different. Again, the results clearly show that the murine *Sts* gene is not subject to normal X-inactivation.

Dosage studies in humans indicate that the *Sts* gene is only partially compensated as the ratio of XX:XO STS activity is consistently less than 2:1 [8–10].

This partial dosage effect was explained by clonal studies which showed that when the Sts^+ allele is on the inactive X, the STS activity of the clone is always less than clones in which the Sts^+ allele is on the active X chromosome [11]. Thus, the Sts^+ allele is partially repressed on the inactive X chromosome. This differs from our results in the mouse where clonal studies show a marked overlap in STS activities between clones with the Sts^+ allele on the active X and those with the Sts^+ allele on the inactive X chromosome.

In humans, besides *Sts*, *MIC2* and *Xg* also have been mapped to the pairing region [17–21] and are not subject to complete X-inactivation [22, 23]. *Sts* and *Xg* show strict X-linked inheritance and therefore do not have Y-linked alleles. *MIC2* does have a functional Y-linked allele but inheritance patterns indicate that recombination does not take place at this locus [24]. Recently, two anonymous human DNA sequences with Y- and X-linked alleles have been mapped to the pairing region and inheritance patterns indicate that obligatory recombination occurs between them during male meiosis [25–27].

The partial inactivation pattern of the human *Sts*, *Xg*, and *MIC2* genes and their lack of recombination with Y-linked alleles could be of considerable interest. Perhaps genes in the pairing region in man are in an evolutionary transit from an ancestral state of functional X- and Y-linked alleles with crossing over distal to the region of recombination to one of true X-linked inheritance proximal to the region of recombination. In this ancestral state, genes distal to the region of obligatory recombination must retain functional X- and Y-linked alleles or a nonfunctional allele would soon be transferred to the X chromosome and a high frequency of males and females with two nonfunctioning alleles would result. Since functional X- and Y-linked alleles are present in this region, both X-linked alleles must remain active to maintain equal dosage in males and females. In humans, no expressed genes have been detected in the true or recombinational pairing region. Conceivably, the region may now have only DNA sequences that function in pairing and recombination to which the two "anonymous" sequences recently found belong, while genes formerly in this region are evolving to a simple X-linked X-inactivation condition.

The mechanism that maintains genes in the pairing region in an active state is unknown. We know from two different experiments with *Sxr* that this gene becomes subject to inactivation after it is transposed to the pairing region [6, 7]. Its normal location on the Y chromosome presumably does not involve inactivation. On the other hand, the murine *Sts* that normally resides in the pairing region is not subject to X-inactivation. These results suggest that the signal for resistance to X-inactivation involves the gene and not the region, in which case, the signals may still be present in genes such as *Xg*, *Sts*, and *MIC2* in humans even though they are no longer distal to a region of obligatory recombination.

In conclusion, we have shown both by dosage studies and by measuring expression of the *Sts* gene on the active and inactive X chromosomes that *Sts* in the mouse is not inactivated. Furthermore, we suggest that the lack of X-inactivation in the pairing region may be simply related to an ancestral situation

where functional X- and Y-linked alleles were distal to a region of obligatory recombination.

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