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X-inactivation of the *Sts* locus in the mouse: an anomaly of the dosage compensation mechanism

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Summary

The behaviour of the *X*- and *Y*-borne *Sts* locus has been studied in male and female mice. There was considerable heterogeneity in STS activity between inbred mouse strains, with a four fold difference in activity between the highest (101/H) and lowest (Ju/Ct) activity strains, which can be interpreted in terms of allelic differences. In all inbred strains male STS levels were higher than those of female STS levels and in the majority of strains tested male STS levels were nearly twice as high as female levels. Reciprocal crosses between C3H/HeH and the STS-deficient substrain, C3H/An, demonstrated that activities of the *X*- and *Y*-borne genes in males are essentially the same and this suggested that the lower STS level in females derives from *X*-inactivation of the locus. The possibility that hormonal differences could instead be responsible for the lower activity in females was ruled out by the findings that (*a*) castration of males did not reduce their STS levels and (*b*) sex-reversed males, *X/X Sxr*, had STS levels typical of females. Final proof that the mouse *Sts* locus can be subject to the *X*-inactivation process was provided by the observation that *XX* females had STS levels that were only slightly (20%) higher than those of *XO* females. The difference may indicate incomplete inactivation of the locus. Linkage data verifying the location of *Sts* on the distal end of the *X* chromosome are provided.

In total, the results of this study show that the murine *Sts* locus can be subject to the *X*-inactivation process and this, together with the existence of functional loci of near-equal activities on the *X* and *Y* chromosomes, results in an imbalance of STS levels between the sexes. *X*-inactivation does not therefore serve as a dosage compensation mechanism for the *Sts* locus in the mouse. All of these findings were made in C3H/HeH mice or in animals carrying C3H/HeH functional *Sts* alleles, and it is pointed out that the diverse results previously obtained by other investigators may be attributable to their use of different strains and crosses between strains but could also be complicated by technical factors.

1. Introduction

Recent molecular and genetic evidence from man and the mouse has indicated that the *X* and *Y* chromosomes share a small region of homology at which pairing occurs. Obligatory crossing-over in this region during male meiosis results in the exchange of loci between the *X* and *Y* chromosomes (Evans *et al.* 1982; Cooke *et al.* 1985; Simmler *et al.* 1985; Rouyer *et al.* 1986) with the consequence that distally located loci do not appear to be sex linked. Rather, they show a pseudoautosomal pattern of inheritance (Burgoyne, 1982).

The gene for the microsomal enzyme, steroid sulphatase (STS – EC 3.1.6.2) in the mouse was initially thought to be autosomally inherited (Erickson *et al.* 1983; Keinanan *et al.* 1983). However, the discovery that oocytes from *XO* females have only half the STS levels of oocytes from *XX* females (Gartler & Rivest, 1983), which have both *X* chromosomes in the active state, and that STS-deficient males transmitted a null *Sts* allele to their *XO* daughters via the *X* chromosome (Keitges *et al.* 1985) clearly indicated that the gene was located on the *X*. On the basis of linkage in females with the distal visible marker, cream (*Crm*) (Cattanach & Crocker, 1986) the *Sts* locus has since been assigned to the distal end of the *X*.

Other genetic studies have shown that a functional *Sts* locus is also carried on the *Y* chromosome (Keitges *et al.* 1985) and linkage has been demon-

strated with the sex-reversed mutation (*Sxr*) (Nagamine *et al.* 1987; Keitges *et al.* 1987) that is located at the distal end of the long arm of the *Y* chromosome beyond the pairing region (Evans *et al.* 1982). This, coupled with the apparent autosomal pattern of inheritance has confirmed that *Sts* is located within the pseudoautosomal region (Craig & Tolley, 1986).

It is not clear from the published data so far available whether the *Sts* locus is subject to *X*-inactivation. Gartler & Rivest (1983) initially reported that *XO* females had STS levels in somatic tissues that differed little from those of *XX* females, suggesting that *Sts* is subject to the *X*-inactivation process. On the other hand the same group later reported that *XX* females have higher STS levels than *XO* females (Keitges & Gartler, 1986) and concluded that *Sts* is not subject to *X*-inactivation. This is surprising since the more distally located *Sxr* transposition appears to be *X*-inactivated (Cattanach *et al.* 1982; McLaren & Monk, 1982). Furthermore, the many reports of essentially equal STS activities in males and females (Crocker & Craig, 1983; Erickson *et al.* 1983; Keinanan *et al.* 1983; Keitges *et al.* 1985; Keitges & Gartler, 1986), could also support an escape from *X*-inactivation if it is assumed that males have two full doses of the *Sts* gene (Keitges & Gartler, 1986). However, again discordant results have been obtained. Lam *et al.* (1983) reported that male mice have STS levels twice those of females and this has also recently been found in the root vole (Wiberg & Fredga, 1987). Such higher STS levels in males might argue that the locus is inactivated in females.

Comparison of the mouse *Sts* locus with its human homologue, *STS*, is complicated by the fact that in humans the *X* chromosomal locus lies outside the pseudoautosomal region (Geller *et al.* 1986) and a functional *Y*-borne locus does not exist (Fraser *et al.* 1987). Human *STS*, therefore, shows an *X*-linked inheritance. Studies on somatic cell hybrids and cloned cells indicated that alleles on both *X* chromosomes were expressed (for a review see Shapiro, 1985; Mohandas *et al.* 1980; Shapiro *et al.* 1979) but quantitative studies on cloned fibroblasts have since established that on the inactive *X* chromosome this locus is only partially expressed and thus that there is incomplete *X*-inactivation (Migeon *et al.* 1982).

Preliminary investigations in this laboratory have indicated that the ratio of STS activities in *XO* and *XX* female mice is 1:1·4 and suggested that the locus was inactivated (Jones *et al.* 1988). However, as these experiments proceeded the results were found to vary among family groups in a way that suggested that *Sts* alleles of different activities could be segregating. In our new studies, reported here, we have investigated the STS activities in a number of inbred strains and found strain differences in STS activity which could confound genetic studies. We have further studied *Sts* inactivation and dosage compensation in one selected strain, C3H/HeH. The results provide clear evidence that in C3H/HeH females the *Sts* locus is subject to *X*-inactivation, and because of the equal activities of the *X*- and *Y*-borne loci in males there is an imbalance of STS enzyme activities between the sexes.

2. Materials and methods

(i) *STS assays*

Routine STS assays were performed on 100 mg of liver from mice at 5 weeks of age. However, for diagnostic purposes on animals which were required for subsequent breeding a single testes was removed from males under Avertin anaesthesia. In both cases the tissue was homogenized in 2 ml 0·1 M-HEPES (pH 8·0) sonicated for 45 s and then centrifuged at 12000 *g* for 15 min. All assays were performed in triplicate on 0·18 ml supernatant plus 0·02 ml [6,7-^{3}H(N)]oestrone sulphate (NEN diluted to a specific activity of 2·5 mM, 74 MBq/mmol). Following incubation at 37 °C for 30 min the reaction was terminated by the addition of 0·1 ml 1·0 M-NaOH and free [^{3}H]oestrone was extracted twice with toluene (1:1 v/v). The radioactivity recovered in the combined organic phases was determined by scintillation counting. Under these conditions STS was kinetically saturated ($K_m = 60\ \mu$m) and the C3H/HeH liver extracts gave a linear response with protein concentration and time. STS assays were performed on 60 animals at a time, which included controls to ensure that assays were consistent. In all experiments STS levels were determined with appropriate controls but data from different assays could be meaningfully combined (results not shown). The expressed units of STS have been corrected for the blank control (prepared by the addition of NaOH prior to the substrate) and are expressed as nmol h^{-1} mg $protein^{-1}$, determined using the biuret method (Boehringer, Mannheim) (mean ± S.E.M.).

(ii) *Statistical analysis*

Unless otherwise indicated all statistical comparisons have used the Mann–Whitney *U* test or *F* test from analysis of variance.

3. Mice

(i) *Inbred strains and crosses*

STS levels were determined in male and female mice of the inbred strains 101/H, 129/Sv, BALB/cNimr, C57BL/6J, SWR/Ola, C3H/HeH, CBA/H, JU/Ct and C3H/An and those of the (C3H/HeH × 101/H) and (C3H/HeH × C3H/An) F_1 hybrids. The STS-deficient C3H/An substrain was kindly provided by Dr J. L. Guenet of the Pasteur Institute, Paris.

The null *Sts* allele carried by the C3H/An strain is

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here denoted Sts^n and that of the functional allele of the C3H/HeH strain Sts^a.

(ii) *Linkage of Sts and Li*

In order to verify that the *Sts* locus studied was located on the *X* chromosome, linkage with the visible distal *X* chromosome marker lined (*Li*) (Cattanach, 1985) was tested. The *Li* gene is maintained in a stock of mixed C3H/HeH – 101/H genetic background and therefore *Li*/+ females can carry *Sts* alleles (here arbitrarily called Sts^+) of either or both strains. The *Li* $Sts^+/+Sts^+$ females were crossed with STS-deficient males, carrying the Sts^n allele, to produce *Li*/+ females of genotype *Li* $Sts^+/+Sts^n$. These females were then backcrossed to STS-deficient males and evidence of *STS-Li* linkage sought among the wild-type male progeny. Non-recombinants ($X\,Sts^n/Y\,Sts^n$) would therefore be the STS-deficient and recombinants ($X\,Sts^+/Y\,Sts^n$) would show STS enzyme activity.

(iii) *Non-specific hormonal influences on STS activity*

To test for the effect of endogenous sex hormones male C3H/HeH mice were castrated, the STS levels determined in their livers 12 days post-operatively, and the results compared with those from uncastrated C3H/HeH male controls.

STS levels in chromosomal *X*/*X* mice which were phenotypically male through the possession of the *Sxr* sex-reversing factor (*X*/*X Sxr*) were also compared with *X*/*Y* male and *X*/*X* female sibs as a further test of sex hormone influences. For this study *X*/*Y Sxr* male mice from the *Sxr* stock, which is maintained on a mixed C3H/HeH and 101/H genetic background, were first selected for STS levels typical of C3H/HeH males on the basis of STS expression in testes and were then mated with C3H/HeH females to produce *X*/*X Sxr*, *X*/*Y Sxr* and *X*/*Y* male and *X*/*X* female progeny. All test animals should therefore have carried the Sts^a allele on both sex chromosomes.

(iv) *Activities of* X- *and* Y-*borne loci*

X- and *Y*-borne *Sts* gene activities were determined by comparing the STS levels of *X*/*Y* males derived from reciprocal crosses between C3H/HeH and C3H/An mice. One group therefore carried the null allele on the *X* while the other carried the null allele on the *Y*. STS levels were also determined in reciprocal cross F_1 females and compared with levels in their male sibs and inbred C3H/HeH females.

(v) *Sts inactivation*

In order to investigate whether the *Sts* locus was subject to *X*-inactivation in females, *XO* mice of mixed C3H/HeH and 101/H background, and carrying the visible marker tabby (*Ta*) on their single *X* chromosome, were crossed with C3H/HeH males. The STS levels of their wild-type (*X*/*O*) daughters, which should carry the Sts^a allele, were compared with those of C3H/HeH (*XX*) females.

4. Results

Li causes non-random *X*-chromosome expression in heterozygotes and pre-natal lethality in the male (Cattanach & Crocker, 1984), therefore linkage was determined only through the analysis of wild-type male progeny of the crosses. The data are presented in Table 1 and show that *Sts* and *Li* are tightly linked in females. Of 65 wild-type males tested, 4 recombinants ($X\,Sts^+/Y\,Sts^n$) were identified by the presence of STS activity (5·7, 6·6, 7·2 and 7·9 nmol h^{-1} mg $protein^{-1}$). The remaining 61 males tested were STS deficient, non-recombinants ($X\,Sts^n/Y\,Sts^n$) that had activities typical of C3H/An males ($0{\cdot}47 \pm 0{\cdot}04$ nmol h^{-1} mg $protein^{-1}$, not corrected for background). The estimate of recombination between *Sts* and *Li* was $6{\cdot}1 \pm 0{\cdot}3$ %. The data therefore not only verify that the STS levels measured were attributable to a sex chromosomal *Sts* locus but provide further evidence for the location of the *Sts* gene at the distal end of the *X* chromosome.

Table 2 presents information on STS activities in male and female mice of a number of inbred strains and the C3H/HeH × 101/H cross. Two key observations should be noted. First, there was a wide range of STS levels among the inbred strains tested; a fourfold difference was found between the highest (101/H) and the lowest (JU/Ct) activity strains. In addition, the F_1 hybrid between low (C3H/HeH) and high (101/H) activity strains exhibited STS levels which were intermediate between those of the parental strains. These findings confirm and extend the observations of others (Erickson *et al.* 1983; Keinanen *et al.* 1983) and on the basis of the studies on recombinant inbred strains (Erickson *et al.* 1983) may be interpreted in terms of allelic differences at the *Sts* locus.

Table 1. *Linkage of* Sts *and* Li *in female mice*

Recombination between *Sts* and *Li* Parental mating *Li* $Sts^+/+ \times +\,Sts^n/Y\,Sts^n$	No. tested
Non-recombinant $+\,Sts^n/Y\,Sts^n$	61
Recombinant $+\,Sts^+/Y\,Sts^n$	4
Total	65 (R.F. = $6{\cdot}1 \pm 0{\cdot}3$ %)

Sts^+ is a high activity functional *Sts* allele carried by *Li* females.
C3H/An males had STS activities which were not greater than those of blank controls. All non-recombinants also had activities which were equal to blank controls (< 250 cpm) and differed from those of recombinants which had activities of approx. 1000 cpm in all replicate samples.

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Table 2. *STS levels in male and female mice from a number of inbred strains and the F_1 hybrid (C3H/HeH × 101/H)*

Inbred strain or cross	STS activity (nmol h^{-1} mg $protein^{-1}$) Females	Males	Male: female ratio of STS activities
101/H	14·31 ± 1·38 (9)	23·13 ± 1·63 (9)	1:0·62*
129/Sv	12·84 ± 0·90 (12)	21·78 ± 1·74 (5)	1:0·59*
BALB/cNimr	14·07 ± 0·92 (12)	18·47 ± 1·74 (12)	1:0·76**
C57BL/6J	11·88 ± 0·65 (13)	16·44 ± 1·57 (12)	1:0·72**
SWR/Ola	8·19 ± 0·60 (4)	13·68 ± 1·64 (4)	1:0·60*
C3H/HeH	6·35 ± 0·19 (30)	11·16 ± 0·44 (20)	1:0·57*
CBA/CaH	4·94 ± 0·66 (5)	7·95 ± 0·66 (6)	1:0·62*
JU/Ct	2·95 ± 0·38 (10)	5·46 ± 0·30 (5)	1:0·54*
C3H/HeH × 101/H F_1	10·90 ± 1·12 (13)	19·13 ± 1·84 (12)	1:0·57*

* The male:female ratios of these strains are homogeneous [$F(6, 144) = 0·22$; $P = 0·97$]. The mean male:female ratios are $1:0.58 \pm 0·0025$, which differ significantly from a ratio of 1:0·5 ($t_{144} = 3·32$; $P = 0·0011$).
** The BALB/cNimr and C57Bl/6J strains have an average male:female ratio of $1:0·75 \pm 0·05$ which differs significantly from the mean of the remaining strains. (*) ($t_{75} = 3.2$; $P = 0·016$).

Table 3. *The relationship between STS activity and sex chromosome composition in male mice*

Strain or cross	Genotype studied	STS activity (nmol h^{-1} mg $protein^{-1}$)
C3H/HeH ♂***	*X* Sts^a/*Y* Sts^a	11·16 ± 0·44 (20)
C3H/HeH ♂ castrated	*X* Sts^a/*Y* Sts^a	10·76 ± 0·36 (11)
C3H/HeH × *X/Y Sxr***	*X* Sts^a/*Y* Sts^a *Sxr*?*	11·26 ± 0·8 (7)
	X Sts^a/*X* Sts^a *Sxr**	6·93 ± 0·45 (6)
	X Sts^a/*X* Sts^a*	6·95 ± 0·92 (2)
C3H/HeH ♀***	*X* Sts^a/*X* Sts^a	6·35 ± 0·19 (30)

* These animals are offspring from the same mating. The STS level in the liver of the *X/Y Sxr* father** was typical of a C3H/HeH male (11·18 nmol h^{-1} mg $protein^{-1}$).
*** Data from Table 2.

The second pertinent observation from Table 2 is that male STS levels were consistently found to be higher than those of females, although in two strains (BALB/cNimr and C57BL/6J) the sex differences were less pronounced. In the other six strains tested (101/H; 129 Sv; SWR/Ola; C3H/HeH; CBA/Ca and Ju/Ct) the male:female ratios were remarkably homogeneous [$F(6, 144) = 0·22$; $P = 0·97$] and male STS levels were nearly twice those of females (average male:female ratio, $1:0·58 \pm 0·025$). This average male:female ratio accords well with the higher male STS levels recently reported in the root vole (Wiberg & Fredga, 1987) and with some early mouse data (Lam *et al.* 1983). Elevated STS levels in males may be considered a first line of evidence to indicate that the *Sts* locus could be subject to *X*-inactivation in females. However, this conclusion would only be valid if male–female STS differences do not merely reflect non-specific hormonal differences and if the activity of the gene is the same when on the *X* and *Y* chromosome. These questions have been addressed only in mice carrying the C3H/HeH and C3H/An *Sts* alleles (Sts^a and Sts^n respectively).

Non-specific endogenous sex-hormonal influences in C3H/HeH males can be excluded as the cause of the male–female difference by two findings. First, as shown in Table 3, castration did not affect the STS levels relative to uncastrated control males (11·26 ± 0·8 and 11·16 ± 0·44 nmol min^{-1} mg $protein^{-1}$ respectively [$P = 0·84$]). Secondly, the STS levels in *X/X Sxr* male mice were typical of female STS values (6·93 ± 0·15 and 6·95 ± 0·92 nmol min^{-1} mg $protein^{-1}$ respectively) and hence much lower than their *XY* male sibs (Table 3). Taken together the two sets of data indicate that the higher STS levels in males reflect a sex chromosomal difference.

Evidence that the higher male STS levels were not attributable to the *Y*-borne locus having a higher activity than the *X*-borne locus was provided by the results of reciprocal crosses involving the STS deficient

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Table 4. *The STS activities in male and female mice with a single functional* Sts^a *allele*

	Strain or cross	Genotype	STS activity
Males with a single functional Sts^a allele			
X-borne	C3H/HeH × C3H/An	$X\ Sts^a/Y\ Sts^n$	$5{\cdot}75 \pm 0{\cdot}3$ ($n = 20$)
Y-borne	C3H/An × C3H/HeH	$X\ Sts^n/Y\ Sts^a$	$6{\cdot}02 \pm 0{\cdot}38$ ($n = 22$)
Females with a single functional Sts^a allele			
Heterozygous females	C3H/An × C3H/HeH C3H/HeH × C3H/An	$X\ Sts^n/X\ Sts^a$ $X\ Sts^a/X\ Sts^n$	$3{\cdot}72 \pm 0{\cdot}14$* ($n = 53$)
XO females	Ta/O × C3H/HeH	$X\ Sts^a/O$	$5{\cdot}28 \pm 0{\cdot}36$ ($n = 18$)
Females with two functional Sts^a alleles**	C3H/HeH	$X\ Sts^a/X\ Sts^a$	$6{\cdot}35 \pm 0{\cdot}19$ ($n = 28$)

* The STS levels of heterozygous female mice ($3{\cdot}77 \pm 0{\cdot}2$ and $3{\cdot}69 \pm 0{\cdot}19$) were indistinguishable ($P = 0{\cdot}86$) and have been pooled.
** Data from Table 2.

substrain C3H/An. These data (Table 4) establish that males with a *Y*-borne functional Sts^a allele, and the null allele on their *X*, have similar STS levels to males who possess the functional allele on their *X* and the null allele on their *Y* ($6{\cdot}02 \pm 0{\cdot}38$ *vs.* $5{\cdot}75 \pm 0{\cdot}3$ nmol h^{-1} mg protein^{-1} respectively, these levels were not significantly different [$P = 0{\cdot}5$]). The presence of STS activity in $X\ Sts^n/Y\ Sts^a$ males not only confirms the existence of a functional allele on the *Y* chromosome but, more importantly, it establishes that this locus has the same activity as that on the *X* chromosome. It may be concluded that C3H/HeH males will have two equal doses of the *Sts* gene and hence the lower STS levels generally found in females might well be attributed to *X*-inactivation. This conclusion is supported by the further observation that C3H/HeH females have STS levels which are similar to those of males with a single functional Sts^a allele (Table 4).

Table 4 also shows that the STS levels of female mice heterozygous for the Sts^n allele ($X\ Sts^{n/a}/X\ Sts^{a/n}$) are lower than those of C3H/HeH females ($3{\cdot}72 \pm 0{\cdot}14$ *vs.* $6{\cdot}35 \pm 0{\cdot}19$ nmol h^{-1} mg protein^{-1}), although higher than the 50% level which might have been expected ($P = 0{\cdot}013$), the observed STS activity ratio being 1:0·58. Furthermore, the STS levels of female mice heterozygous for the Sts^n allele are found to be lower than those of males with a single functional Sts^a allele ($X\ Sts^a/Y\ Sts^n$ and $X\ Sts^n/Y\ Sts^a$). These data are consistent with heterozygous females having a single Sts^a allele which is inactivated in approximately half of the somatic cells.

Further evidence that the *Sts* locus is subject to *X*-inactivation in C3H/HeH mice is provided by the finding that females with two functional Sts^a alleles had STS activities that were similar to those of females possessing a single C3H/HeH *X* chromosome (*XO*) and hence a single Sts^a allele (Table 4). However, the observed STS levels in the *XX* females were 20% higher than those of *XO* females (ratio *XO*:*XX*, 1:1·2 [$P = 0{\cdot}013$]) which may perhaps be attributed to incomplete inactivation of this locus in females.

5. Discussion

Much of the published data on the expression of the *Sts* locus in the mouse are contradictory and this could be attributed to a number of factors. Firstly, in some previous experiments animals derived from crosses between a variety of strains were used. From the strain differences in STS activities presented here (Table 2) it is evident that recombination between the sex chromosomes may result in the segregation of *Sts* alleles and this could create inconsistent results if mice which are heterogeneous with respect to the origin of their *X* and *Y* chromosomes are used. Evidence of segregation of *Sts* alleles was apparent in our early studies on STS levels in *XO* and *XX* females which were on mixed (C3H/HeH and 101/H) genetic backgrounds (Jones *et al.* 1988). In that study the segregation of STS activities resulted in *XO*:*XX* ratios that varied among family groups (1:1·1, 1:1·4, 1:1·4, 1:1·7 and 1:2·2). Consequently, although the mean *XO*:*XX* ratio of 1:1·4 suggested that the *Sts* locus was subject to inactivation, if incompletely, the data from the individual groups could have supported complete, partial or an escape from inactivation of the locus.

Secondly, strain specific variation in male:female STS activities could account for some inconsistencies in the published results on the behaviour of the *Sts* locus. In all the inbred strains studied here male STS levels were found to be greater than those of females (Table 2), and this clearly at variance with reports of equal STS activities by others (Crocker & Craig, 1983; Erickson *et al.* 1983; Keinanen *et al.* 1983; Keitges & Gartler, 1986). However, the extent of the sex difference found in the present study varied between the strains, being less evident in two strains, BALB/cNimr and C57BL/6J.

Finally, the suggestion that the STS assay is not always linear with protein concentration (Keitges & Gartler, 1986) implies that in such experiments the enzyme was not kinetically saturated, and this would influence the apparent STS levels. In the present study

the STS assay was performed under kinetically saturating conditions and thus was independent of protein concentration, and was shown to be specific for a sex chromosomal gene product. In addition, the critical work on the inactivation of the *X*-borne *Sts* locus and the *X*- and *Y*-borne gene activity has been confined to mice which carried the functional Sts^a alleles of the C3H/HeH strain. A high degree of confidence can therefore be attached to the results obtained.

The data obtained indicate that the pseudoautosomal *Sts* locus is subject to *X*-inactivation in C3H/HeH females. The most conclusive evidence for this was the similar STS levels in *XO* and *XX* females (Table 4). Further evidence is provided by the finding that *XX* females of the C3H/HeH strain have lower STS activities than the males but similar activities to those of males with a single functional STS allele ($X\ Sts^a/Y\ Sts^n$ or $X\ Sts^n/Y\ Sts^a$). The latter were also approximately half those of males with two functional STS alleles. However, the data also provides some indication that inactivation of the locus is incomplete. The ratio of STS activities in *XO* and *XX* females departed slightly, but significantly, from equality (1:1·2, $P = 0{\cdot}013$). Furthermore, in the C3H/An mouse strain, the male:female activity ratios (Table 2) also departed slightly, and significantly ($P = 0{\cdot}025$) from the ratio 1:0·5 (*XY*:*XX*) expected with complete inactivation in females and the existence of two functional loci in males. The data are therefore consistent with partial *X*-inactivation of the *Sts* locus in the mouse. At a cellular level incomplete *X*-inactivation could arise by the *Sts* allele on the inactive *X* chromosome being fully functional in a small proportion of the cells (20%) or by a 20% expression of the *Sts* allele when on the inactive *X* relative to that of the active *X* chromosomes. Whether partial inactivation of the *Sts* locus is clonal or due to a uniform repression of activity could help to explain the variable expression of male or female phenotypic sex in *X*: autosome translocations which also carry *Sxr* on the intact and inactive *X* chromosome (Cattanach *et al.* 1982; McLaren & Monk, 1982). Clonal studies such as those used to demonstrate that the human *STS* locus is differentially expressed on active and active *X* chromosome (Migeon *et al.* 1982) should help to resolve this question in mice.

In humans, females have higher STS levels than males (Migeon *et al.* 1982) the reverse of that reported here for the C3H/HeH mouse. In humans, males do not have a functional *STS* locus on the *Y* chromosome, which is consistent with the location of the locus outside the pseudoautosomal region on the *X*. The higher activity in females results from partial activity of the *STS* allele on the inactive *X* chromosome (Migeon *et al.* 1982). Therefore, incomplete *X*-inactivation of the human *STS* locus results in partial dosage compensation. On the other hand, the mouse *Sts* locus lies within the pseudoautosomal region and there is a functional homologue on the *Y* chromosome. In order to achieve dosage compensation one would therefore expect the *X*-borne locus to escape from *X*-inactivation (Lyon, 1961). However, in the C3H/HeH mouse, and possibly the other mouse strains, there is *X*-inactivation of the *X*-borne *Sts* locus despite the presence of a functional *Y*-borne locus in males. This provides a novel situation in which *X*-inactivation does not lead to dosage compensation but results in an imbalance of STS activities between the sexes. A similar phenomenon may also exist in the root vole, which, like the mouse, has a higher STS activity in males than females (Wiberg & Fredga, 1987). However, it may also be significant that the extent of the imbalance in STS levels between the sexes varied among inbred mouse strains (Table 2), and was less pronounced in two strains. This could indicate strain differences in the extent of inactivation of the locus although non-specific biochemical or hormonal differences could be responsible. Either mechanism may help to explain the observed variation of *Sts* expression in the mouse (Crocker & Craig, 1983; Erickson *et al.* 1983; Gartler & Rivest, 1983; Keitges *et al.* 1985; Keitges & Gartler, 1986; Lam *et al.* 1983). To address this issue a further study of *Sts* expression in strains showing less pronounced male:female differences in STS activity is in progress.

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