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SHORT PAPER

Steroid sulphatase in the mouse

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SUMMARY

A form of the human skin disease, ichthyosis, results from a mutation at the steroid sulphatase locus (*STS*) on the *X* chromosome. This locus appears to escape inactivation in the *XX* female, resulting in the expression of two doses of the STS gene (Shapiro *et al.* 1978; Crawfurd, 1982). The scurfy mutation in the mouse is thought to be homologous to the human disease (McKusick, 1978), and so should also be due to a steroid sulphatase (STS) deficiency. Our findings in male and female mice suggest that, in contrast to the human, the murine '*STS*' locus is subject to *X* chromosome inactivation. However, another interpretation of the results is possible, namely that STS may be coded for by an autosomal gene.

1. INTRODUCTION

One of us has recently reviewed (Polani, 1982) facts and hypotheses concerning genes on the human *X* chromosome which seem to escape inactivation, including *STS*, and has proposed that they may be contained and ranked in position within the pairing segment of the *X* chromosome with the *Y*, as determined by electron microscopy (Solari, 1980). It is suggested that they escape inactivation because of their position. This may relate them to the ancestral autosome which, during Man's evolution, was transformed into a sex chromosome. It is also suggested that *STS* may be at the proximal end of the pairing segment (see below). In Man, the ratio of STS (E.C.3 . 1 . 6 . 2) activity in females compared with males (used as the criterion for escape of inactivation) falls short of the 2:1 predicted on grounds of gene dosage. This we attribute to a heterochromatic position effect, such as that well documented in *Drosophila*, for example (Lewis, 1950). This would be in keeping with the experimental observations of Migeon *et al.* (1982). These authors consider alternative explanations for their findings, but a variable spread of inactivation into *STS* would fit well with the position that we visualize for this locus in Man, at the boundary of the pairing segment on the *X* chromosome. However, other explanations could underlie the observations on STS dosage ratio (see Discussion).

If *scurfy* (*sf*) in the mouse is, as alleged, homologous to *X*-linked ichthyosis, it should depend on a mutation of a steroid sulphatase gene ('*STS*'), which should be on the murine *X* chromosome, at *sf*. Such a view is in keeping with the idea that *X*-linked genes are 'conserved' on the *X* chromosomes of all mammals (Ohno, 1969). However, the *sf* locus is close to the centromere (Roderick & Davisson, 1982) and, being distant from its pairing segment, '*STS*' in the female mouse should undergo random inactivation if the views proposed above are correct (Polani, 1982). Thus it would behave differently in this

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mammal compared to Man (Mohandas *et al.* 1980; Müller *et al.* 1980), and a rodent, the Wood Lemming (Ropers & Wiberg, 1982).

In this study, we have accepted the view that the murine scurfy phenotype is homologous to X-linked ichthyosis. The murine and human X-linked STSs should therefore also be homologous, but clearly confirmation of this is required.

2. MATERIALS AND METHODS

Steroid sulphatase and α-galactosidase (as a control) were measured in two different tissues from two different age groups. In these experiments we used C3H mice. They were sexed by phenotype. Primary fibroblast cultures were established in F 10 with 20% foetal calf serum, from limb buds and tails of 20 d 14 and d 16 foetal mice (day of plug = 1).

Table 1. *Steroid sulphatase activity in the mouse*

	Steroid sulphatase activity (pmole/mg protein/h; *foetal livers* per 16 h)			
	Male*	Female*	Significance of sex difference†	Male:female ratio
Foetal fibroblasts	278·3 ± 145·1 (n = 10)	174·9 ± 47·9 (n = 10)	$P > 0·05$	1·59
Foetal liver (whole homogenate)	95·3 ± 28·6 (n = 8)	44·86 ± 75·9 (n = 7)	$P < 0·01$	2·12
Adult liver (whole homogenate)	237·8 ± 80·2 (n = 10)	174·5 ± 62·9 (n = 10)	$P > 0·05$	1·36
Adult liver (microsomal fraction)	518·2 ± 182 (n = 10)	444·4 ± 145·5 (n = 10)	$P > 0·5$	1·17

* Mean ± s.d. † Statistical analysis by Student's t test.

All tests were done at passage 6. Cells were harvested at comparable confluency with EDTA, washed twice with normal saline; enzymes were extracted in lysis buffer (for details, see Ropers *et al.* 1981) by sonication using an MSE Ultrasonic Disintegrator (2 × 30 sec bursts at low power). STS activity was assayed using dehydroepiandrosterone sulphate as substrate (Ropers *et al.* 1981). Ether extraction was done in 1 ml; 500 μl of ether phase was evaporated to dryness and the residue dissolved in 10 ml Aquasol. Radioactivity was counted in a Beckman LS 350 scintillation counter. Each sample was tested in duplicate. Protein was assayed by the Lowry method.

Livers dissected from 20 5-week-old mice and from 15 d-16 foetal mice were homogenized in 0·3 mm-sucrose. In adults only, microsomal fractions were also isolated (Iwamori *et al.* 1976). STS activity was determined in both the whole liver homogenates and in liver microsomal fractions (adults only), employing standard 2 h incubation conditions (Ropers *et al.* 1981), but foetal liver samples were incubated for 16 h.

Total α-galactosidase activity was measured at the same time for all the samples except adult liver microsomal fractions (Fensom *et al.* 1979). We were unable to maintain adult mouse fibroblast sufficiently long in culture for biochemical tests.

3. RESULTS

Table 1 shows that, for the two different tissues and age groups examined, the males always have a higher mean STS activity than the females, but the sex differences are

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not statistically significant, except for the liver of d 16 foetal mice, in which all the 8 males expressed STS activity, while 5 out of the 7 females did not. This we attribute to differential growth and maturation of the two sexes. The foetal liver STS activity is also much lower than its adult counterpart, probably another aspect of developmental differences. In the different tissues and age groups studied, an overall male:female ratio of 1·26 can be obtained from the above data for mean STS activity. On the other hand, α-galactosidase activity, as an example of the product of an X-linked gene known to be inactivated (Lusis & West, 1976), showed a nearly 1:1 sex ratio.

Table 2. *α-Galactosidase activity in the mouse*

	α-Galactosidase activity (nmol/mg protein/h)			
	Male*	Female*	Significance of sex difference†	Male:female activity ratio
Foetal fibroblast	611 ± 166 ($n = 10$)	601·5 ± 224 ($n = 10$)	$P > 0·5$	1·02
Foetal liver (whole homogenate)	43·29 ± 3·41 ($n = 8$)	45·99 ± 4·8 ($n = 7$)	$P > 0·2$	0·94
Adult liver (whole homogenate)	34·19 ± 4·7 ($n = 10$)	35·3 ± 4·2 ($n = 10$)	$P > 0·5$	0·97

* Mean ± S.D. † Statistical analysis by Student's t test.

4. DISCUSSION

The STS findings, and a comparison with the data on α-galactosidase, run counter to the possibility that in female mice '*STS*' behaves as if it escaped inactivation. However, this does not exclude the possibility that the specific *STS* gene may not be X-linked in the mouse. Given that we are dealing with the same locus in Man, the mouse, and – most importantly – in another rodent, the Wood Lemming, then the mouse would be especially interesting because it would be an example of the breaking of Ohno's Law (Ohno, 1969), and the splitting of an allegedly conserved linkage group. This possibility we are attempting to test at present. Clearly, were this the case, then the 'scurfy' phenotype, that cannot, for genetic reasons, be attributed to a deficiency of an autosomal STS, would depend on another STS enzyme/protein, and may not be an exact homologue for X-linked ichthyosis in Man. Or, indeed, it may not be a homologue at all, and may have nothing to do with STS. Alternatively, and of equal interest, the mouse '*STS*', if X-linked, would be involved in inactivation as suggested above. This would not be surprising if '*STS*' is at the *sf* locus, or at least outside the pairing segment of X and Y, and this is also being investigated at present.

From the experiments, the dosage situation in the mouse is in fact almost the reverse of that in Man, the male tending to higher STS activity than the female in the tissues tested. If further work continues to show a higher STS activity in males, the question would then arise, why is this so? A not too implausible suggestion might be that the effect is in some way due to testosterone and its metabolism. This possibility is at present being explored, but receives some support from our findings in mouse foetuses of the two sexes. It could be that similar considerations apply to the human male, and that this may be the reason for the divergence of the male:female ratio from the expected 1:2, in spite

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of *STS* escaping lyonization. It is perhaps worth noting that many males with *X*-linked ichthyosis have undescended testes (Traupe & Ropers, 1982), which suggests the possibility that their testosterone activity or metabolism may be faulty. However, the findings of Migeon *et al.* (1982) offer a completely different explanation for the observed ratio, based on the behaviour of STS when coded by its gene on the inactive *X*, and we may be observing a heterochromatic position effect.

Finally, should the results of further work in the mouse confirm the idea that '*STS*' is *X*-linked, and especially if it mapped at *sf*, the results would also support the idea that genes on the *X* chromosome of mammals escape inactivation when located in the pairing segment of the *X* and *Y* chromosomes, and may not inactivate autonomously.

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