Lack of evidence that inactivation of the mouse X-chromosome is incomplete

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1. INTRODUCTION

Russell (1963, 1964) has suggested that inactivation of the second X-chromosome of female mice (*Mus musculus* L.) is incomplete. She took as evidence for this suggestion, on the one hand, the lack of inactivation effects in some X-Autosome translocations and, on the other hand, the absence of any visible heterozygous effect, especially a mosaic effect, in female mice heterozygous for the genes scurfy, *sf*, or sparse-fur, *spf*. She postulated that the loci of these two genes were in a part of the X that was not inactivated. Recent evidence (Cattanach & Isaacson, 1965) indicates that these loci are close together on the side of *Bn* (bent-tail) away from *Ta* (tabby) and hence are at the end of the linkage group.

As far as the X-Autosome translocations are concerned, evidence from another mouse translocation (Lyon *et al.*, 1964), and from structural abnormalities of the human X, suggests that the presence of a translocation may interfere with the normal inactivation process. Therefore, although a lack of inactivation in some of these translocations may provide valuable evidence as to how inactivation occurs, it does not show that the X-chromosome regions concerned remain active, in a chromosomally normal animal.

There remains the question of the lack of mosaic effect in females heterozygous for scurfy or sparse-fur. Failure of an X-linked gene to show a heterozygous effect is not conclusive evidence that it does not undergo inactivation, as there are various reasons why a gene expected to show mosaicism may fail to do so (Lyon, 1966*a*). One possibility is that although the two types of cells, with either the mutant or the normal gene active, may be present in equal numbers in the early embryo, when inactivation first occurs, one type may outgrow the other during development, so that only one type of cell is present in the adult. Other possibilities are that gene product may diffuse from cell to cell, or that the visible end-result of a gene may be due to a non-localized or non-autonomous action, so that there is no macroscopic patchy effect in the adult, although two types of cell are in fact present. Each of these kinds of masking of the mosaic effect depends on the random inactivation of one or other of the two X's in different cells of the same animal, and inactivation at the locus concerned should therefore become apparent in any animal in which the same X was active in all cells. This is the situation in mice heterozygous for Searle's

translocation, T(X; ?)16H. Lyon *et al.* (1964) showed that in females carrying this translocation there was non-random X-inactivation, and the X involved in the translocation remained active in all cells whilst the normal X was inactive. Thus, no mosaic effects were seen, but instead, genes on the translocated X were fully expressed as in a male, whereas those on the normal X were not expressed at all.

Thus, if the genes scurfy or sparse-fur were being inactivated it should be possible to demonstrate the fact by introducing them into females carrying Searle's translocation. A heterozygote with the mutant allele on the active chromosome should then show the full expression of the mutant, with the wild-type allele inactive. The experiment is unfortunately impracticable with scurfy, since scurfy males do not breed and T16H males are sterile, but the present paper describes the results of such a test with sparse-fur.

2. PLAN OF EXPERIMENT

Heterozygous females of the type T16H $+ / + Ta$ or T16H $+ / + Blo$ were crossed with *spf* males. The phenotypically non-tabby or non-blotchy daughters, which were expected to be genetically $+ T16H/spf +$ were mated to $+ Ta +$ males, and the young were classified for *spf* and *Ta* (Tabby). From this cross females of the type *spf* T16H $+ / + + Ta$ were expected to arise by crossing-over. (Following Cattanach & Isaacson (1965) and Lyon (1966b) the *spf* locus is thought to lie on the side of the T16H break away from *Ta*, and hence the order of loci is *spf*–T16H break–*Ta*–*Blo*.) If the $+^{spf}$ allele on the normal (non-translocated) X-chromosome was being inactivated these females should show full phenotypic expression of sparse-fur as in *spf spf* or *spf* Y. Any female offspring of this phenotype were kept and genetically tested. To give a control, the occurrence of offspring of this phenotype was also noted in the preliminary crosses, T16H $+ / + Ta \times spf$ and in crosses of $+ / + \female \times spf \male$ in which no translocation was present. The male offspring of the $+ T16H/spf + \times Ta$ cross were classified for T16H on the basis of their testis weight at 5–6 weeks (T16H males having small testes), in order to obtain a measure of the recombination of T16H with *spf*. This recombination was then compared with the frequency of phenotypically sparse-fur females. The genetic test made of these sparse-fur females was to cross them to blotchy (*Blo*) males and to classify male and female offspring for *spf*, *Ta* and T16H as before.

3. RESULTS

From the control crosses of normal female by *spf* male there were no phenotypically sparse-fur daughters (Table 1), confirming that $+/spf$ heterozygotes do not normally manifest the full effects of *spf*. There was one sparse-fur daughter from the preliminary crosses of T16H $+ / + Blo \times spf$ male, which also showed the colour pattern of blotchy and proved on genetic test to be $+ Blo/spf +$. Thus, $+/spf$ females also carrying another X-linked gene may occasionally show full expression of *spf*. Among the offspring of $+ T16H/spf + \times Ta$ crosses, on the other hand, there were sixteen phenotypically sparse-fur daughters, one of which is shown in Plate I.

Phenotypically sparse-fur female (centre) of genotype *spf* T16H + /+ +*Ta*, with a sparse-fur male (right) and a normal male from the same litter. The female's pheno-type is indistinguishable from that of the sparse-fur male.

Table 1. *The frequency of phenotypically sparse-fur daughters among the offspring of control and test crosses. (Tx signifies the translocation T16H)*

Parents		Phenotypes of female offspring		
♀	♂	Normal	Sparse-fur	Tabby or blotchy
$\dfrac{+\ +\ +\ +}{+\ +\ +}$	$spf+++$	27	0	—
$\left.\begin{array}{l}\dfrac{+\ Tx\ +\ +}{+\ +\ Ta\ +}\ or \\[2mm] \dfrac{+\ Tx\ +\ \ +}{+\ +\ +\ Blo}\end{array}\right\}$	$spf+++$	12	1	11
$\dfrac{+\ Tx\ +\ +}{spf\ +\ +\ +}$	$++Ta+$	34	16	65

The first six of these sixteen to have been genetically tested have all proved to be carrying both *Ta* and T16H. Therefore, since *spf* and T16H were carried by one parent and *Ta* by the other, the genotype of these daughters must have been *spf* T16H + / + + *Ta*. This shows that females heterozygous for *spf* can show the full expression of this gene if it is carried on the T16H chromosome. There remains the question whether all females of this genotype are phenotypically sparse-fur. These females occurred as a result of crossing-over between *spf* and the T16H translocation break. Among the male offspring from the same crosses the *spf*–T16H recombination was measured as $23 \cdot 0 \pm 4 \cdot 5\%$ (Table 2). If the sparse-fur female offspring

Table 2. *Data on recombination between the T16H break and the* spf *locus, provided by male and female offspring. (Tx signifies the translocation T16H)*

Parents		Phenotypes of offspring						
		Female			Male			
♀	♂	$+Tx$	$spfTx$	$++Ta/+++$*	$+Tx$	$spfTx$	$++$	$spf+$
$\dfrac{+\ Tx\ +\ +}{spf\ +\ +\ +}$	$++Ta+$	34	16	65	40	9	11	27
$\dfrac{spf\ Tx\ +\ +}{+\ +\ Ta\ +}$	$+++Blo$	5	8	25	8	13	6†	1†

* These animals may or may not have carried *spf*
† These animals also carried *Ta*.

represented the whole of the *spf* T16H + / + + *Ta* crossover class then the recombination among the female offspring was $32 \cdot 0 \pm 6 \cdot 6\%$, in fairly good agreement with the value from the males. Furthermore, confirmation of this point was provided by the offspring of the genetically tested *spf* T16H + / + + *Ta* animals. Again, phenotypically sparse-fur daughters were found, which in this case should represent the

non-crossover class, and again the recombination values from the male and female offspring were in good agreement (male, $32 \cdot 1 \pm 8 \cdot 8\%$; female, $38 \cdot 5 \pm 13 \cdot 5\%$) if the sparse-fur daughters were taken to represent the whole of the non-crossover class.

Thus, the evidence suggests that all females carrying *spf* on the T16H chromosome show the full expression of *spf*. The implication is that the $+^{spf}$ allele on the normal X-chromosome was genetically inactive in all cells. Therefore, one would expect that in a genetically $+$ T16H/*spf*$+$ animal the *spf* allele would be genetically inactive. Although chromosomally normal $+$/*spf* females do not show the full expression of *spf*, some of them do show a mild expression. Six out of twenty-seven $+$/*spf* offspring of $+/+ \times spf$ crosses showed such expression, together with eleven out of twenty-seven $+$/*spf* in crosses of $+$/*spf* $\times spf$. By contrast, among the offspring of T16H $+/+ Ta$ or T16H $+/+ Blo \times spf$ none out of twelve $+$T16H/*spf*$+$ females showed any expression of *spf*, although three out of the eleven $+Ta$/*spf*$+$ or $+Blo$/*spf*$+$ offspring did so. This is as would be expected if the *spf* allele in the $+$T16H/*spf*$+$ females was genetically inactive in all cells.

4. DISCUSSION

Evidence has been presented that in a genetically *spf* T16H/$+ +$ female the normal allele of *spf*, on the structurally normal X-chromosome, is genetically inactive. This is taken to show that the part of the mouse X-chromosome in which the *spf* locus lies does take part in the inactivation process. It is important that, in the experiments described here, it was the allele on the structurally normal X that was inactive. Since this X had not been involved in any chromosome rearrangements there is no reason to suppose that any kind of position effect could have interfered with the behaviour of the genes on it, and every reason to think that if the *spf* locus in the normal X of a T16H heterozygote undergoes inactivation then it would also do so in the X-chromosomes of a chromosomally normal animal.

There is, in fact, no evidence that any region of the mouse X is not inactive in a chromosomally normal animal. Russell (1963) has presented evidence that, in female mice heterozygous for X-Autosome translocations, some segments of the translocated X may not show inactivation, and in other segments the spread of inactivation from the X into the attached autosomal segments may be limited. As mentioned earlier, however, this provides no evidence of the behaviour of an intact X. If there is some inactivation centre (as suggested by Grumbach (1964), Lyon (1964) and Russell (1964)) from which the process spreads, or more than one, as suggested by Cattanach & Isaacson (1965), then in an intact X the inactivation may well spread the entire length of the chromosome, whereas a segment of X separated from the inactivation centre or centres by translocation to an autosome may fail to undergo inactivation.

Similarly, although there are some loci, including those of *sf*, *Gy* (gyro) and *jp* (jimpy), for which it is not possible to obtain conclusive evidence of inactivation, owing to the technical difficulties imposed by death or sterility of the males, there is on the other hand no evidence to suggest that they are not inactivated, and the

appearance of gyro and of jimpy heterozygotes is consistent with that which would be expected if they were inactivated.

Conversely, the fact that a chromosomally XO mouse (Welshons & Russell, 1959) is so phenotypically normal provides positive evidence suggesting that the whole of the second X of XX female mice is indeed inactive. Cattanach (1962) made a detailed study of XO mice and showed that their postnatal viability and growth up to 3 weeks of age were equal to those of XX mice. Human XO's, on the other hand, show the definite abnormalities of Turner's syndrome (Ford *et al.*, 1959; Lindsten, 1963) and many die prenatally (Carr, 1965). Various possible explanations can be put forward for this departure of human XO's from the expected consequences of the inactive-X hypothesis. Ferguson-Smith (1965) studied the phenotypic effects in human females in which the second X was not entirely lacking, as in XO, but was partially deleted. He concluded that the results could be explained by postulating that a part of the short arm remained active in both X's of the human female and was homologous with a region of the Y. It would be consistent with the dosage compensating effect of X-inactivation that such a pairing segment should not undergo inactivation (Lyon, 1962, 1966*a*). The evidence thus suggests species differences in the completeness of X-inactivation. In man a small region remains active, in the mouse inactivation is complete, and in the Oregon vole (*Microtus oregoni*) the process has been taken to the extreme of actual loss of the inactive X (Ohno, 1963). Similar variations appear to occur in marsupials (Hayman & Martin, 1965*a*, *b*).

Another entirely different point brought out by the present work concerns the mode of action of the *spf* gene. The absence of a mosaic effect in $+/spf$ heterozygotes when one or other X is being inactivated at random could be due to (*a*) unequal life-spans or rates of formation of cells with the $+$ or the *spf* allele active, or (*b*) very small size of patches of normal or mutant cells, or (*c*) a non-localized action of the *spf* gene. Since other genes affecting hair texture, such as tabby (*Ta*) and striated (*Str*), produce easily visible patches, the second possibility seems most unlikely. The first explanation again seems unlikely, particularly since a mild, though non-mosaic, heterozygous effect of *spf* can sometimes be seen. This leaves as the most likely explanation a non-localized action of the *spf* gene, i.e. the observed effect of *spf* is probably due not to the action of a gene product produced within the hair follicle cells themselves, but to that of some substance which diffuses from elsewhere. This probability should form the basis of any further studies of the action of *spf*, and of the action of any other X-linked genes which can be demonstrated to undergo inactivation but do not produce a mosaic effect in heterozygotes. Thus, Grüneberg (1966) claims that the abnormalities of the teeth of tabby heterozygotes are not as would be expected from X-inactivation. The tabby locus is known to undergo inactivation, however, both from the mosaic patches in the coat of heterozygotes and from the full phenotypic expression of the allele on the active X in heterozygotes carrying Searle's translocation. Therefore, the effects in the teeth can best be explained by postulating that the growth of tooth cusps does not depend on the autonomous action of individual precursor cells for each tooth, but on the

combined action of groups of cells, common to the animal's whole dentition. One obvious possibility is that the shape of the tooth cusps depends on the head mesenchyme, which from its very loose structure and migration during development, will not have the discrete patches of cells descended from a single precursor that are found in epithelial tissues, but will instead show quantitative variation from animal to animal in the proportion of cells with the mutant allele active. Thus the effects in the teeth of tabby heterozygotes provide a pointer to further studies of the action of the tabby gene.

SUMMARY

The fact that the X-linked genes scurfy (*sf*) and sparse-fur (*spf*) of the mouse do not produce a mosaic effect in heterozygotes had been taken, by other workers, together with results from X-Autosome translocations, as evidence that inactivation of the mouse X was incomplete. In this paper it is argued that absence of a mosaic effect is not adequate evidence that a gene is not inactivated. The argument was backed by an experiment in which the *spf* gene was introduced heterozygously into females carrying an X-linked translocation resulting in non-random X-inactivation with the same X active in all cells. When the mutant (*spf*) allele was on the active X its effect was fully expressed, indicating that the normal allele on the structurally normal inactive X was undergoing inactivation. Argument is further presented that results from X-Autosome translocations do not indicate the degree of completeness of inactivation in a structurally normal X. Hence, there is no evidence that inactivation of the mouse X is incomplete, although evidence from XO females does suggest that it may be incomplete in man.

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