Age-related reactivation of an X-linked gene close to the inactivation centre in the mouse

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Summary

Age-related reactivation of an X-linked gene which maps close to Xce, the X chromosome inactivation centre, has been observed. In five female mice which carried the X-linked coat colour gene Mo^{blo} on the reciprocal translocation T(X;16)16H (Searle's translocation), and the wild-type gene on the normal X chromosome, and therefore expressed the Mo^{blo} phenotype due to the non-random inactivation characteristic of Searle's translocation, progressive darkening of the coat was observed as the animals aged. This is due to reactivation of the previously inactivated wild-type gene at the Mo locus on the normal X chromosome. As the Mo locus is located 4 cM distal to Xce, the X chromosome inactivation centre, these observations provide evidence of age-related instability of inactivation of an X-linked gene close to the inactivation centre.

1. Introduction

In female mammals one of the two X chromosomes is genetically inactivated, resulting in dosage compensation for X-linked genes (Lyon, 1961). The onset of X-chromosome inactivation in embryogenesis is linked to cellular differentiation, occurring in a random fashion in the precursor cells of the embryo proper just prior to gastrulation (reviewed in Monk, 1981; Gartler & Riggs, 1983). The X chromosome controlling element, (Xce), locus affects the randomness of inactivation (Cattanach & Isaacson, 1967; Cattanach *et al.* 1969; Rastan, 1982) and is located in the region of the X chromosome which has been shown functionally to contain the X-chromosome inactivation centre (Russell & Cacheiro, 1978; Rastan, 1983; Rastan & Robertson, 1985). X-inactivation is stable and heritable in somatic cells; in female germ cells, however, there is reactivation of the previously inactivated X-chromosome just before the onset of meiosis (Johnston, 1981). Of a variety of experimental treatments used to attempt to reactivate the inactive X chromosome in somatic cells, only a few have proved successful. Treatment with 5 azacytidine, a demethylating agent, can cause re-expression of individual X-linked genes (e.g. Mohandas *et al.* 1981; Venolia *et al.* 1982; reviewed in Monk, 1986) and Takagi *et al.* (1983) achieved reactivation of a previously inactive X-chromosome in thymocytes of the mouse by fusing

them with mouse EC cells to form near-tetraploid hybrids. In addition, Migeon *et al.* (1985) have described expression of the $G6PD$ locus on the inactive X chromosome in cultured cells derived from human chorionic villi, and have subsequently described complete reactivation of the previously inactive X-chromosome in cultured cells derived from human chorionic villi after fusion with mouse A9 fibroblasts to form interspecific hybrid cells (Migeon *et al.* 1986).

There is also evidence that autosomal loci which are involved in X-autosome translocations, and inactivated by virtue of their proximity to inactivated X chromosomal material (Russell & Montgomery, 1970), may subsequently escape inactivation (Cattanach, 1974), and recently age-related reactivation of a true X-linked gene, sparse fur (*spf*), located at the proximal tip of the X chromosome, a considerable distance from the inactivation centre, has been demonstrated (Wareham *et al.* 1987). In this paper we describe age-related reactivation of another true X-linked gene, at the Mo locus, close to the inactivation centre.

2. Mice

Five female mice heterozygous for T(X;16)16H (hereafter abbreviated to T16H) in which the Mo^{blo} gene has crossed-over onto the translocated X(16*) have been produced in our breeding colony.

T16H is a reciprocal translocation between the X

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chromosome and chromosome 16 (Fig. 1) which results in marked non-random X-chromosome inactivation (Lyon *et al.* 1964). In females heterozygous for the translocation the normal X is inactivated in all cells. In our laboratory T16H was maintained in matings using two X-linked mutant marker genes, Mo^{blo} (Mottled) (Green, 1981) and Ta (Tabby) (Green, 1981). Fig. 2 shows the breeding system used. Wild-type females carrying T16H and either Mo^{blo} or Ta on the normal X chromosome were mated to either TaY or $Mo^{blo}Y$ (light coat) males, and generated $Mo^{blo}Y$ (light coat) or TaY males, Mo^{blo}/Ta females with a
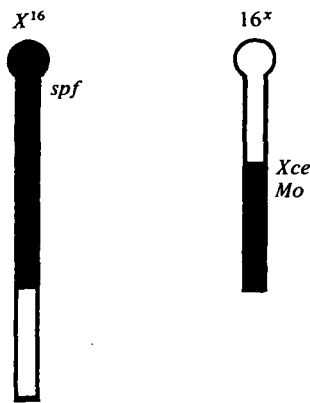
characteristic pale striped phenotype, sterile T16HY males (wild type) and females which were heterozygous for T16H and carried either Ta or Mo^{blo} on their normal X chromosome (i.e. T16H $++/+Ta+$ or T16H $++/++Mo^{blo}$), and which were wild type due to non-random inactivation of the normal X chromosome.

Atypical females with a Mo^{blo} phenotype, having the same coat colour as $Mo^{blo}Y$ male litter-mates, arose in three separate matings from our breeding colony. One mating, T2X/60 produced three females

Fig. 1. Diagram of T(X;16)16H, Searle's translocation, showing the relative positions of *spf*, *Xce* and *Mo*.

T16H++/++Mo^{blo} × TaY
(wild type ♀)   (Tabby ♂)

|

T16H++/+Ta+ ; ++Mo^{blo}/+Ta+ ; T16HY ; Mo^{blo} Y
(wild type ♀)   (pale, striped ♀)   (wild type,   (Blotchy ♂)
                                     sterile ♂)

or

T16H++/+Ta+ × Mo^{blo} Y
(wild type ♀)   (Blotchy ♂)

|

T16++/++Mo^{blo} ; +Ta+/++Mo^{blo} ; T16HY ; TaY
(wild type ♀)   (pale, striped ♀)   (wild type, (Tabby ♂)
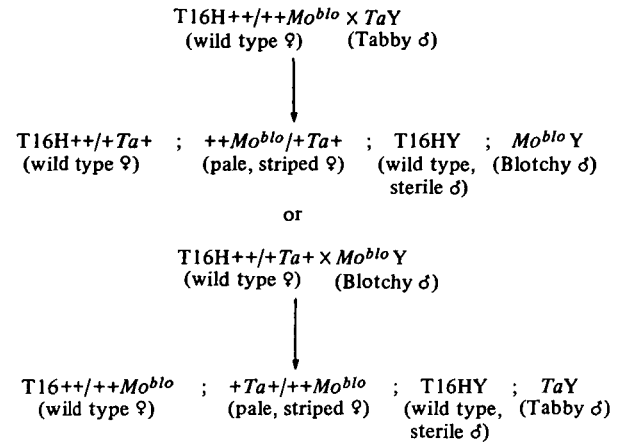   sterile ♂)

Fig. 2. Breeding scheme used to maintain T16H (Searle's translocation).

Fig. 3. Metaphase chromosome spread from peripheral blood culture of a Mo^{blo} female showing a normal diploid karyotype.

(a) Female i aged 7 months

(b) Female i aged 9 months

(c) Female ii aged 9 months

(d) $Mo^{blo}Y$ male control aged 8 months

FIGURE 4. Coat colour of two T16H+Mo^{blo}/+Ta+ females showing darkened coat compared to a $Mo^{blo}Y$ male control.

with a Mo^{blo} phenotype, one of which died before further investigation could be carried out. Matings T2X/81 and T2X/82 each produced one Mo^{blo} female. Karyotypic analysis from peripheral blood cultures showed a normal chromosome constitution of 40 acrocentrics, (Fig. 3) thus excluding an XO constitution as the cause of the Mo^{blo} phenotype. An alternative explanation is that the Mo^{blo} gene has crossed-over onto the translocated X chromosome (16^x), and is therefore non-randomly expressed in all cells. This was confirmed by progeny testing. All the Mo^{blo} females were mated to TaY males. If they were of the hypothesized T16H + Mo^{blo}/ + Ta+ genotype they would be expected to produce TaY males, Ta/Ta females, T16HMo^{blo}Y sterile males (light coat) and females with a Mo^{blo} phenotype heterozygous for T16H and Ta (T16H + Mo^{blo}/ + Ta+). The offspring produced were as expected, and in addition two wild-type sterile males were produced. These would have been produced by the Mo^{blo} gene crossing-over back onto the normal X, leading to inheritance of a translocated X carrying the wild-type gene. A female with a Mo^{blo} phenotype produced from the progeny test was herself mated to a TaY male, and also produced the expected offspring.

Changes in the coat colour of T16H + Mo^{blo}/ + Ta+ females began to be seen at around six months of age. Areas of the coat where the wild-type gene was reactivated expressed wild-type coat colour leading to progressive patchy darkening of the coat as the animals aged. A gradual progressive darkening of the coat was observed in all the females, although the extent of darkening varied between individuals. Figure 4 shows females at seven months and nine months of age compared to an adult Mo^{blo}Y control male.

3. Discussion

In five T16H + Mo^{blo}/ + Ta+ females, age-related reactivation of the wild-type allele at the Mo locus carried on the previously inactivated normal X was observed. Areas of the coat where the wild-type gene was reactivated expressed the wild-type coat colour, leading to progressive darkening of the coat as the animals aged.

The fact that three T16H + Mo^{blo}/ + Ta+ females arose from one mating implies that a single cross-over event occurred during an early mitotic division of one of the cells giving rise to the germ line of the mother. This would lead to a clone of germ cells in which Mo^{blo} was carried on the 16^x translocation product.

Wareham *et al.* (1987) have shown reactivation with age of an X-linked gene, sparse fur (*spf*), which is located at the proximal tip of the X, in females doubly heterozygous for T16H and *spf*. The *spf* gene involves a mutation of the structural gene coding for the urea cycle enzyme ornithine carbamoyl transferase (OCT), leading to a histochemically detectable ab-

normal form of OCT. In *spf* T16H/ + + females the wild-type allele on the previously inactivated normal X was shown to be subject to age-related reactivation in the liver. However, *spf* maps 44 cM proximal to *Xce*, the X chromosome inactivation centre, and in fact is the furthest known gene from the inactivation centre. The inactivation centre is known to mediate the onset of X chromosome inactivation in the female embryo (Russell & Cacheiro, 1978; Johnston & Cattanach, 1981; Rastan, 1982; Rastan, 1983; Rastan & Robertson, 1985); if the inactivation centre is also important in maintenance of X-chromosome inactivation it would seem likely that genes furthest away from the inactivation centre, such as *spf*, would be the ones most likely to escape from X-inactivation as the cell ages. The effect of distance from the inactivation centre on inactivation or otherwise of a gene is exemplified by the spread of inactivation into autosomal material in X-autosome translocations in which genes nearer to the breakpoint in the translocation product carrying the inactivation centre are more likely to be affected than genes further away (Eicher, 1970; Cattanach, 1974), and by the variable sexual phenotype of mice heterozygous for T16H and *Sxr* (Cattanach *et al.* 1982; McLaren & Monk, 1982). *Sxr* involves a piece of the Y chromosome carrying the testis-determining gene(s) translocated to the distal tip of the X chromosome (Evans *et al.* 1982; Burgoyne, 1982; Singh & Jones, 1982). XX*Sxr* animals usually develop as sterile males, but T16HX*Sxr* animals, in which the X*Sxr* chromosome is inactivated in all cells, may develop as fertile females, hermaphrodites or sterile males, depending presumably on the proportion of cells contributing to the primordial gonad with the attached *Sxr* region on the inactive X also inactivated due to the variable spread of inactivation into the *Sxr* material (McLaren, 1986).

The Mo locus, a true X-linked gene, maps only 4 cM distal to *Xce* and is shown in this study to be subject to age-related reactivation as detected in the coat. These observations show that spontaneous reactivation of a true X-linked gene *in vivo* is not necessarily prevented by proximity to the inactivation centre. Furthermore these data on the reactivation of the Mo locus in the melanocytes of the coat, combined with the earlier evidence for the reactivation of the gene coding for OCT in the liver, suggest that there may be a *general* destabilization with age of the maintenance of the X-chromosome inactivation system.

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