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Genetic activity at the albino locus in Cattanach's insertion in the mouse

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SUMMARY

Cattanach's insertion (Is(In7;X)1Ct or X^{Ct}) includes the normal allele at the albino locus (c^+), which is subject to inactivation of the X chromosome carrying it, so that $X^{Ct}X$; *cc* mice have albino and pigmented patches. The X-autosome translocation T(X;16)16H or X^{T16H} leads to preferential inactivation of the other X chromosome in female cells, so that $X^{Ct}X^{T16H}$; *cc* mice are almost entirely white. However, they grow darker with age, as if reversal of inactivation of the c^+ allele were taking place in increasing numbers of melanocytes. To test whether this is dependent only on age or whether it is related to the number of times the animal has moulted, hair was repeatedly plucked from selected areas at the early telogen stage when the follicles are also removed, assuming that the melanocytes or melanoblasts in that region of the skin would be forced to undergo further divisions to colonize the new follicles. The plucked areas grew darker at the same rate as the rest of the coat, suggesting that the progressive reversal of inactivation is dependent only on age.

As direct examination of melanocytes in the follicles is difficult, they were examined in the choroid and the retinal pigment epithelium (RPE) of the eye. The frequency of the pigmented cells was lower in the choroid than in the RPE. Since the melanocytes in these structures are different in origin as well as in physical characteristics, it appears that cell type influences either reversal of inactivation, or the frequency with which the influence of the X chromosome extends to the albino locus.

INTRODUCTION

Cattanach's insertion (Is(In7;X)1Ct or X^{Ct}) consists of a segment of chromosome 7 inserted near the middle of the X chromosome (Cattanach, 1961; Green, 1981). There is evidence that at least some of the genes in the inserted autosomal segment (e.g. c^+, p^+, *sh*-1^+) become subject to inactivation of the X chromosome (Cattanach, 1961; Deol & Green, 1969). For instance, female mice heterozygous for the insertion and homozygous for the gene for albinism ($X^{Ct}X$; *cc*) have both pigmented and albino patches in the coat, depending on whether the c^+ gene in the inserted autosomal segment is active or inactive.

Key words: X-inactivation, mosaics, pigmentation, genetic activity, Cattanach's insertion, albino, mouse, mutation.

It was found that in such animals the albino (*c c*) patches have a strong tendency to grow darker with age, as if there were reversal of inactivation in increasing numbers of melanocytes (Cattanach, 1974). The question arose whether this reversal was dependent on the age of the animal or whether it might be more directly related to the number of cell divisions in the melanocyte lineage. These would increase with the number of moults that the animal had gone through, on the assumption that at each moult the old melanocytes are lost with the old follicle, and the ones colonizing the new follicle arise by cell division from the melanocytes at the dermal–epidermal interface in the vicinity (Billingham & Silvers, 1960). This question could be investigated by repeatedly plucking the hair from clearly delimited areas of the coat at the early telogen stage of the hair cycle, when the follicle, with its melanocytes, generally comes out with the hair. This would force the melanocytes or melanoblasts at the dermal–epidermal interface in that region to divide, and if the darkening of the coat were related to the number of moults, these areas should grow darker at a faster rate than the rest of the coat. Since the effects of plucking, if any, would be easier to identify if the coat were lighter to begin with, another X-autosome translocation (Searle's translocation T(X;16)16H or X^{T16H}) was introduced into the experiment. In female mice heterozygous for this translocation (XX^{T16H}) the translocated X chromosome is preferentially expressed (Lyon, Searle, Ford & Ohno, 1964), so that in $X^{Ct}X^{T16H}$ mice it is the X^{Ct} chromosome which is inactivated (Cattanach, 1974). Since this inactivation includes (in most cells) the translocated autosomal region that carries the normal allele at the albino locus, $X^{Ct}X^{T16H}$; *c c* mice have little pigment in the coat.

As the melanocytes in a hair follicle are difficult to study, and the colour of the hair is only an indirect clue to their function, the pigment cells in the choroid and retina of the eyes of $X^{Ct}X^{T16H}$; *c c* mice were also examined.

MATERIALS AND METHODS

The following schemes were used to obtain $X^{Ct}X^{T16H}$; *c c* mice: (1) XY; *c c* (albino) males were mated with Tabby (*Ta*) XX^{T16H}; + + females, and their non-Tabby daughters, which must be XX^{T16H}; + *c*, were then mated with $X^{Ct}Y$; *c c* (normally pigmented) males. Of the four possible types of daughters only the $X^{Ct}X^{T16H}$; *c c* ones would be almost white, the rest being either normally pigmented or mottled. (2) $X^{Ct}Y$; *c c* males were mated with Tabby XX^{T16H}; + *c* females, and again only $X^{Ct}X^{T16H}$; *c c* daughters would be almost white.

For plucking, the animals were anaesthetized with avertin (0·1 ml/5 g + 0·15 ml, administered intraperitoneally) and placed in the normal resting position. The hair was then removed from areas approximately 1 cm^2 over the right shoulder and the left hip. For the shoulder, the tip of the scapula, felt through the skin, was used as a landmark, and for the hip the tip of the ilium served the same purpose. The plucked hairs were kept and samples examined to ensure that the follicles had been removed. Altogether, 11 mice were used, minimum pluckings being four and maximum seven per mouse. (The average number of moults is about 5–6.) Before and after each plucking the animals were photographed on a standard grey cardboard background beside a normal albino (BALB/c) control animal which was plucked at the same time.

Of the 11 animals used in the plucking experiment, 10 were fixed by perfusion with Wittmaack's fluid for histological examination of the eyes. Their ages ranged from 315–488 days. In addition, six young $X^{Ct}X^{T16H}$; *c c* females (16–28 days old) were fixed to ascertain that age did not affect the pigment of the eye. The eyes, after decalcification and the removal of the lens, were cut out with the surrounding structures *in situ*, and embedded in celloidin followed by

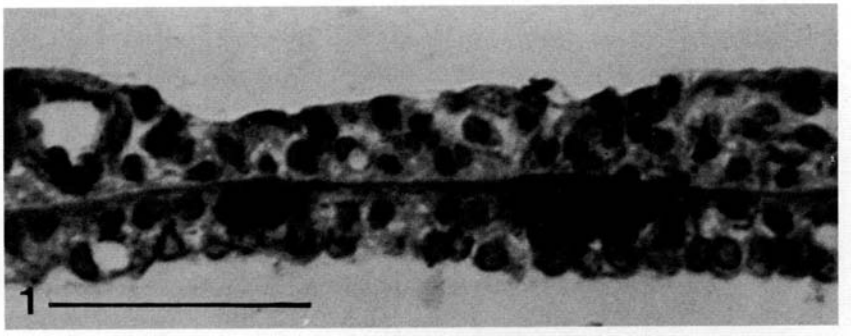


Fig. 1. Transverse section of the iris of a 16-day-old $X^{Ct}X^{T16H}$; *cc* female. Pigmented melanocytes are present in the inner (lower) layer, which is a continuation of the RPE, but they are absent in the outer (top) layer, which is a continuation of the choroid. The border between the two is distinct. Bar, 50 μm.

paraffin (Deol & Truslove, 1981). Serial sections were cut at 10 μm (in some cases at 12½ or 15 μm) and stained in the usual manner.

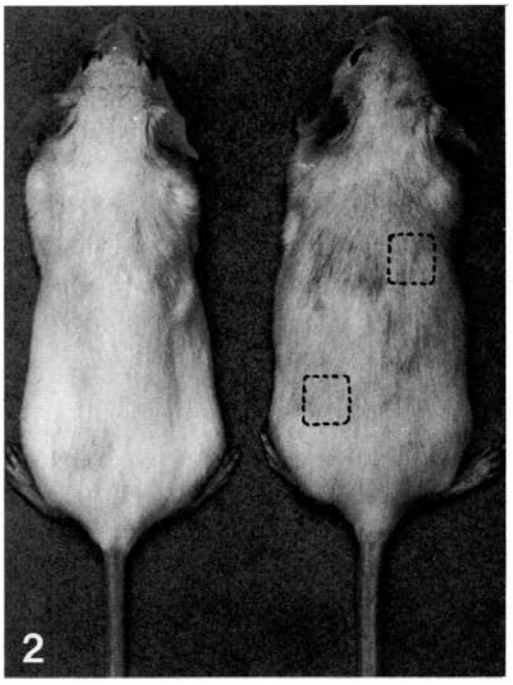
The proportions of pigmented and unpigmented cells in the retinal pigment epithelium (RPE) and especially in the choroid are difficult to determine, but as they are reflected in the inner and outer layers of the iris, respectively, measurements were made on the iris using a Kontron Videoplan Computerized Image Analyser (Reichert-Jung). Both eyes of six $X^{Ct}X^{T16H}$; *cc* females, aged 16–28 days, were used. In each case 10 central sections, 100 μm apart, were chosen. As practically all pigmented regions in both layers touch the border between them (Fig. 1), it was thought that linear measurements along the border, including the total length and the lengths in touch with pigmented areas, would provide a satisfactory estimate of the extent of the pigmented parts.

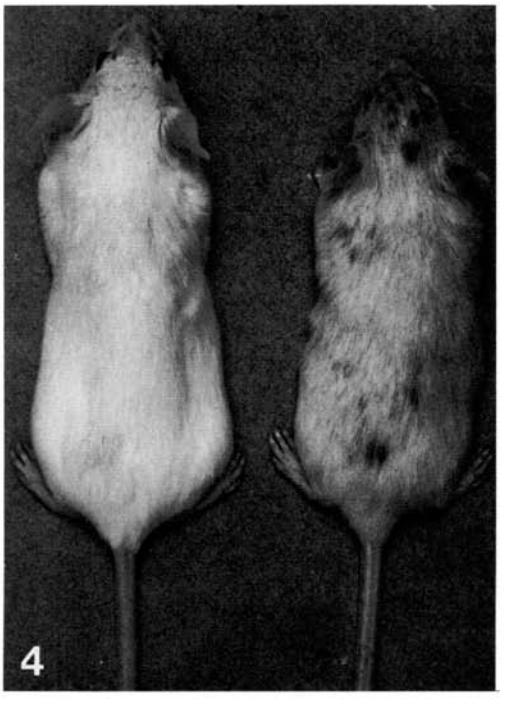
OBSERVATIONS

The first coat in $X^{Ct}X^{T16H}$; *cc* mice was always almost white, although the majority of hairs had some pigment, usually only a trace. Some strikingly dark hairs were also present, occurring singly or in small groups or forming dark spots of irregular size (Figs 2, 4). At each moult the animals grew darker (Fig. 6), though animals that were lighter to begin with remained lighter than others of comparable age. The dark spots did not change much, but grew less and less distinct as the surrounding coat grew darker.

No clear differences in the colour of the coat between plucked and unplucked areas were observed at any stage (Figs 3, 5). Neither did the microscopic examination of the plucked hairs reveal any difference. The plucked areas in the albino (control) animal always became indistinct when the coat had grown to a reasonable length.

Sections of the eyes showed marked differences between the choroid and the RPE, the proportion of pigmented cells being higher in the latter (Fig. 7). Measurements made on the outer and inner layers of the iris (Fig. 1), representing the choroid and the RPE, respectively, in $X^{Ct}X^{T16H}$; *cc* mice are given in Table 1. In general the mice with a higher proportion of pigmented cells in the outer layer also had more pigment in the first coat.





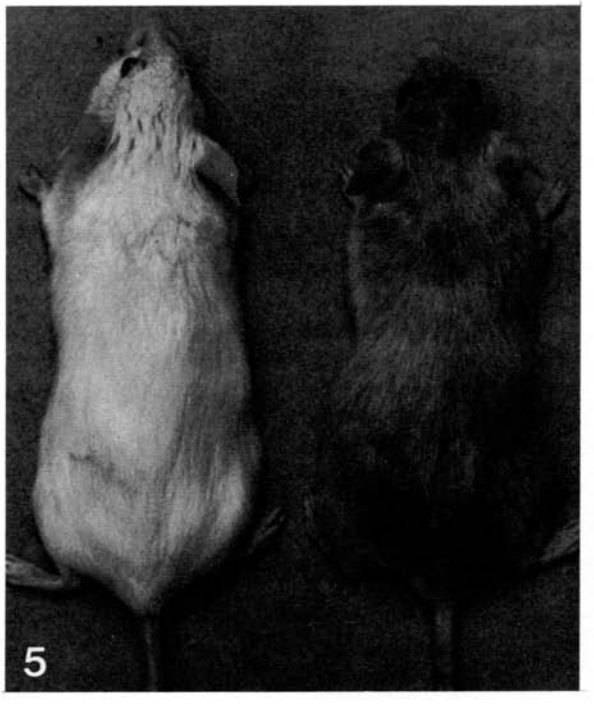


Fig. 2. A 48-day-old $X^{Ct}X^{T16H}$; *cc* female and control (left) before first plucking. Plucking areas outlined by dotted line; note absence of dark spots.

Fig. 3. The same female (and control) as in Fig. 2 at 417 days and after five pluckings. Note the similarity between plucked and unplucked areas.

Fig. 4. A 48-day-old $X^{Ct}X^{T16H}$; *cc* female and control (left) before first plucking. Note dark spots.

Fig. 5. The same female (and control) as in Fig. 4 at 417 days and after five pluckings. Note the similarity between plucked and unplucked areas.

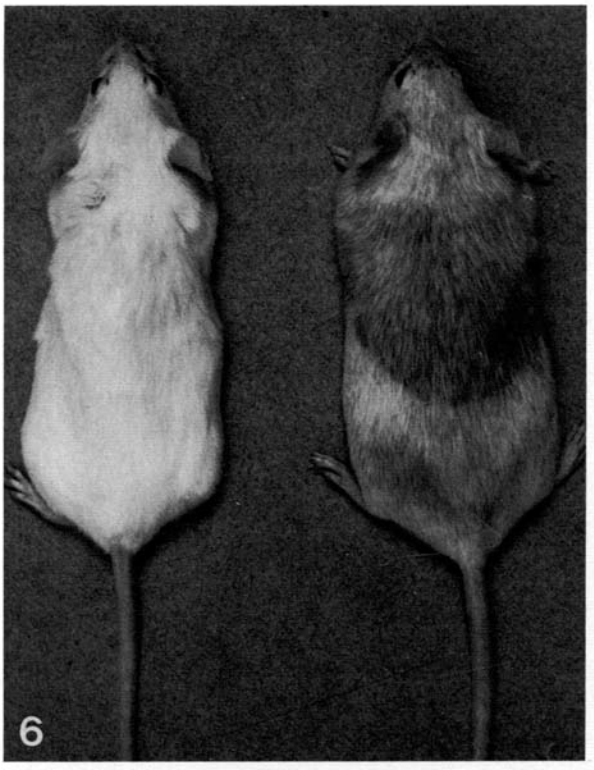


Fig. 6. A 123-day-old $X^{Ct}X^{T16H}$; *c c* female and control (left). Note that the moult lines across the back separate the new and darker hairs from the old and lighter ones.

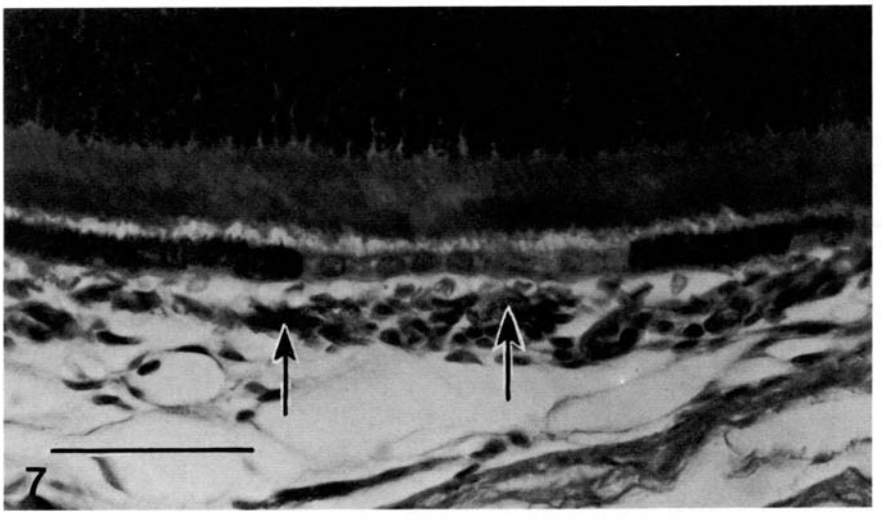


Fig. 7. Transverse section through choroid and RPE of a 16-day-old $X^{Ct}X^{T16H}$; *c c* female. Arrows indicate pigmented patches in the choroid. Bar, 50 μm.

Table 1. *Extent of pigmented parts (in percentages) in the outer and inner layers of the iris in six* $X^{Ct}X^{T16H}$; c c females

	Age (days)	Left		Right		Both	
		outer	inner	outer	inner	outer	inner
	16	3·39	5·36	1·23	5·00	2·34	5·18
	16	0·39	9·20	3·52	13·86	1·92	11·48
	20	7·11	9·16	1·90	11·18	3·75	10·46
	23	0·28	5·92	1·62	10·88	1·13	9·05
	24	15·29	12·83	1·13	9·62	7·74	11·12
	28	3·96	8·57	16·85	9·34	10·15	8·94
mean	21	5·07	8·51	4·38	9·98	4·51	9·37

$t_{10} = 2{\cdot}78$, $P < 0{\cdot}02$.

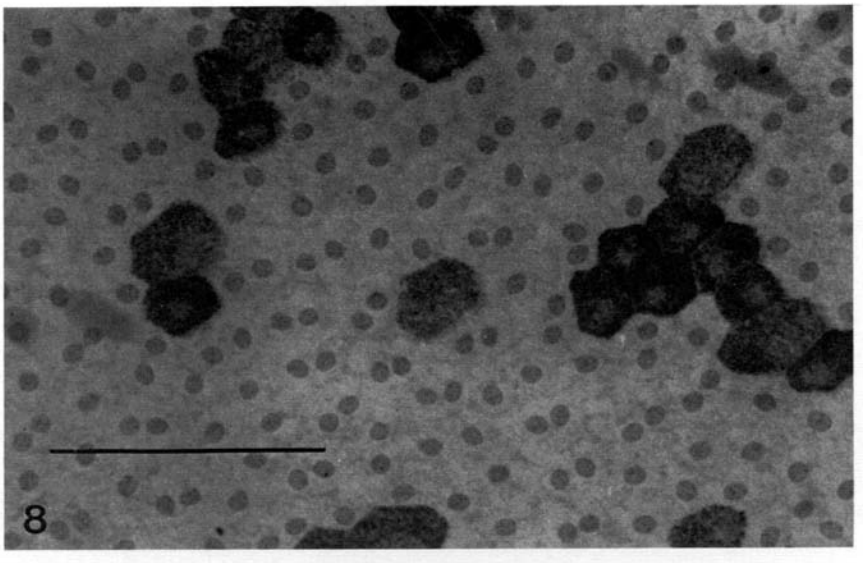


Fig. 8. Tangential section through the RPE of the retina of a 28-day-old $X^{Ct}X^{T16H}$; *c c* female. Bar, 0·1 μm.

There were only two types of cells in the RPE, normally pigmented and entirely unpigmented, and no intermediate ones (Fig. 8). No difference was found between young and old mice in the proportions of the two types of melanocytes in either the choroid or the RPE.

DISCUSSION

Plucking nearly doubles the number of hair cycles, yet it had no obvious effect on the colour of the coat. This suggests that the progressive darkening of the animals, which presumably results from reversal of inactivation of the c^+ gene in the insertion, is a function of the age of the animal, and is not related to the

number of divisions that the melanocytes have undergone. That would also explain why there was no noticeable difference in colour between the plucked hairs and the new hairs, whereas successive moults are strikingly different: as the plucking was done at the early telogen stage of hair growth, when it is most effective, the time interval between the old and new hair growths was much shorter than that between successive moults. The possibility remains, however, that the assumption that the new follicle is colonized by new melanocytes arising by cell division may not be correct.

In contrast to the situation in the hair follicle, there was no age-related change in the frequency of pigmented cells in either the choroid or the RPE. This is not surprising, since these cells change very little throughout life, retaining their pigment once it is formed (Billingham & Silvers, 1960). Indeed, in old $X^{Ct}X^{T16H}$; *cc* mice the pigmentation of the choroid can be regarded as an indication of the colour of the first coat.

While the RPE cells in $X^{Ct}X^{T16H}$; *cc* mice are either pigmented or entirely unpigmented, the great majority of hairs contained some pigment, although generally very little, as also observed by Cattanach & Isaacson (1965). This suggests that colonization of a follicle by a single clone is not a very common event, and that the two types of cells form a fairly intimate mixture in the skin.

The much greater proportion of pigmented melanocytes in the RPE than in the choroid (or in the coat of young animals) is presumably related to the origin and, or, the tissue environments of these cells. The melanocytes in the two structures differ in a number of respects. The choroidal cells, like those of the coat, (a) originate in the neural crest, (b) migrate to their destination, (c) have an irregular shape, with many dendrites, and (d) do not form a regular layer or tissue. Those in the RPE, on the other hand, (a) originate in the primary optic vesicle, (b) do not migrate, (c) have a fairly regular shape, with no dendrites, and (d) form a uniform layer. The melanocytes in the hair follicles are similar to those of the choroid in all respects except that they do not retain their melanosomes, but pass them on to other cells, acting as unicellular glands (Billingham & Silvers, 1960).

Whether the tissue-dependent difference of pigmentation is a matter of absence of inactivation or of its reversal is difficult to say. In one case, the influence of the X chromosome would extend to the albino locus less frequently in the cell lineage that gives rise to the retina; in the other, it would be withdrawn more frequently, the initial situation being the same in the choroid and the RPE.

The relative inactivation frequencies of the two X chromosomes within an individual may vary significantly from tissue to tissue, presumably as a result of small pools of tissue progenitor cells arising as random samples of a larger cell population in which X inactivation has already occurred (McMahon, Fosten & Monk, 1983). However, these tissue differences are not consistent between individuals. Consistent and highly significant tissue differences, between erythrocytes and cultured fibroblasts, have been reported with respect to the X-coded G6PD isozymes (Migeon, 1978; Williams *et al.* 1984). These are believed to be due to cell selection, acting subsequent to X chromosome inactivation. The similar difference

reported here, between the RPE and the tissues that derive their pigment from the neural crest, is unlikely to be due to selection, as there is little or no turnover of cells in these structures, even during the embryonic period, and chimaeras made between pigmented and non-pigmented strains show no consistent difference between the level of pigmentation in the RPE and in the coat (West, 1975). Nor is selection likely to be the cause of the age effect in the coat, as again chimaeras of similar phenotype do not show it (McLaren, 1976). As far as we are aware, the only other such tissue difference known is the trophoblast and tissues derived from the primary endoderm of the mouse (for references see Gartler & Riggs, 1983).

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