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Controlling elements in the mouse *X*-chromosome

I. Interaction with the X-linked genes

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1. INTRODUCTION

Most of the mouse *X*-autosome translocations give rise in the heterozygous female to variegated phenotypes for the autosomal genes attached to the *X* (Cattanach, 1961; Russell & Bangham, 1961; Russell, Bangham & Saylors, 1962). The variegation is attributable to two mechanisms: (1) the inactivation of one or the other *X* in the course of the normal process of *X*-inactivation (Lyon, 1961); and (2) a position effect which appears to be analogous to the V-type position effects described in *Drosophila* (see reviews by Lewis, 1950; Baker, 1968). In effect, when the normal *X* is in its genetically inactive, heterochromatic condition, the autosomal genes attached to the rearranged *X* are able to express their phenotypes. On the other hand, when the rearranged *X* is in the inactive condition there appears to be a spread of the inactivation along a length of the adjoining autosomal material (Cattanach, 1961, 1963; Russell, 1963). Since the spreading effect is variable, the autosomal loci need not always be inactivated when the rearranged *X* is inactivated. Most of the observed variegation appears to be due to the randomness of the *X*-inactivation process, for when this is suppressed such that the rearranged *X* is maintained in its inactive condition in all cells, the autosomal loci are inactive in the majority of these cells. The variegation that remains can be attributed to the position effect mechanism alone (Cattanach, 1966).

In a series of recent experiments with one particular *X*-autosome translocation *T*(1; *X*)*Ct* (Cattanach, 1961), we have been able to show that the position effect variegation is under genetic control (Cattanach & Isaacson, 1965) and that the control rests with an element located in the rearranged *X*-chromosome itself (Cattanach & Isaacson, 1967). From the inheritance of different forms or 'states' of the controlling element it was clear that the element had to be closely linked to the break-point in the *X*. Changes from one 'state' to another were detected and, while it appeared that some of these occurred as a result of a 'change in state' of the element itself such as described in the maize controlling elements systems (McClintock, 1951, 1965), it could not be ruled out that meiotic crossing over was also a responsible mechanism.

Control of position effect variegation by the mouse *X*-chromosome controlling elements implies control of the inactivating properties of the *X*. In so far as the elements appear to operate only upon the *X*-chromosomes in which they are located and only when these chromosomes are in their inactive, heterochromatic

condition, it seemed possible that they might control the inactivation of the *X*-chromosome itself. The present communication describes the results of experiments designed to investigate the possibility. A brief report on part of the data has already been published (Cattanach, 1968).

2. METHODS AND MATERIALS

The translocation, *T*(1; *X*)*Ct*, is one in which a piece of autosome of linkage group I has been inserted into the *X* (Ohno & Cattanach, 1962). In the present experiments all the translocation-bearing animals employed carried the chromosomally unbalanced, duplication form, *Dp*(1; *X*)*Ct*, of the rearrangement. In the translocation lines both normal linkage group I chromosomes carried the coat colour gene *albino* (*c*) and, since the autosomal insertion carries the wild type allele (c^+), the heterozygous female exhibits a *c*-variegated phenotype. The lines were maintained by crossing the heterozygous females or hemizygous males with chromosomally normal *c* animals. For the purposes of this paper, the symbol Dp will be used to describe *Dp*(1; *X*)*Ct* heterozygotes and hemizygotes.

Two states of the controlling element, distinguishable by the levels of *c*-variegation they permit, were used in the present experiments. Both were derived from a single translocation line in which the two 'states' of the element were found to be segregating despite 12 generations of selection for low levels of *c*-variegation (Cattanach & Isaacson, 1967). Each 'state' has subsequently been isolated and then introduced into a common inbred (Ju/Fa) background by way of single progeny-tested Dp males (Cattanach & Isaacson, 1967). Unfortunately, a consequence of establishing the translocation on the inbred background was that the Dp male, which normally exhibits a low viability (Cattanach, 1961), became a lethal class after the second generation of inbreeding. They could, however, be recovered by outcrossing the increasingly inbred females of each generation to an unrelated stock of males. In the experimental crosses to be described both partially inbred and F_1 outcross Dp males were employed. The outcross was made with the use of chromosomally normal *c* males of a *T*(1; *X*) *Ct* line that had been selected over 15 or more generations for a high level of *c*-variegation.

The partially inbred lines and animals carrying the 'states' of the controlling element permitting high and low levels of *c*-variegation have been designated *H* and *L*, respectively, and the equivalent outcross animals, *HX* and *LX*. Numbers associated with these letters, e.g. L_2, refer to the generation of inbreeding (backcrossing to the inbred) of the Dp males themselves, or in the case of the outcross animals, e.g. L_4X, they refer to the generation of inbreeding of mothers. Table 1 summarizes the observations and interpretations of the *c*-variegated phenotypes caused by the 'states' of the controlling element that are carried in the *L* line and *H* line rearranged (X^T) *X*-chromosomes.

The aim of the experiments was to determine whether the controlling elements, in addition to controlling the position effect variegation, also influence the heterozygous expression of the *X*-linked genes. Dp males carrying one or the other of the

two 'states' of the controlling element were crossed with females homozygous or heterozygous for the *X*-linked gene to be studied and the phenotypes of the two types of doubly heterozygous females were then compared. Crosses were also made using the chromosomally normal males from the same lines as the Dp males; these provided control data for any genetic background influences and also tested the *X*-chromosome of the inbred line. Two *X*-linked genes were subjected to test, *Tabby* (*Ta*) and a new allele at the *Mottled* locus that has been given the provisional name of *Viable-brindled* (Mo^{Vbr}).

Table 1. *Observation and interpretations of* c*-variegated phenotypes caused by the low and high 'states' of the controlling element*

Line designation	'State' of X^T chromosome*	Interpretation	*c*-variegation in X^T/X ♀♀	
			Mean amount of *c*	Score
L	Low	c^+ less frequently inactivated when X^T inactivated	Low (aprox. 30 %)	Low
H	High	c^+ more frequently inactivated when X^T inactivated	High (approx. 50 %)	High

* State of controlling element in $T(1; X)Ct$ *X*-chromosomes.

Heterozygous *Ta* females exhibit a variegated or striped coat. The dark stripes contain much of the hair abnormality of the homozygous female or hemizygous male and the remainder of the coat appears normal (Falconer, 1953). *Ta* also reduces the secondary vibrissae number, the reduction being considerably less in the heterozygote than in the homozygote or hemizygote (Dun, 1959; Dun & Frazer, 1959). In order to assess any influence of the controlling elements upon the *Ta*/+ phenotype we elected to score the vibrissae number. This seemed an easier and certainly less subjective criterion for the expression of *Ta* than that of the level of the complex *Ta* banding pattern.

The vibrissae number of 19 is considered to be an invariant character in unselected non-*Ta* mice, but when *Ta* is present, influences due to differences in the genetic background can be detected (Dun & Frazer, 1959). Unfortunately, we have found it impractical to keep our homozygous/hemizygous *Ta* stock on a uniform genetic background; it can, however, be maintained in a healthy, vigorous condition on a mixed (C_3H, 101, CBA) genetic background. In order to control any bias that could be brought into the test-crosses by individual animals, the *Ta* females were circulated amongst the two groups of Dp and chromosomally normal males. A check on any bias brought into the test-crosses from either parent was also provided by the scores of the hemizygous *Ta* male progeny.

Mo^{Vbr}, hereafter abbreviated to *Vbr*, also produces a variegated or striped phenotype in the heterozygous female, the bands being composed of whitish hair like that of the hemizygous *Vbr* male. The gene causes a slight rippling of the coat and a curling of the vibrissae and this suggests that its primary effect may be on

coat structure. The banding seen in the heterozygote is, however, much broader than that of *Ta*/+ animals and it is thus possible that the two genes operate upon different cell types. Alternatively, it is possible that *Vbr* operates upon more than one cell type; certainly a more precise and *Ta*-like banding is seen in animals in which the yellow pigment in the hair is absent, e.g. in *non-agouti* animals.

Vbr males are sterile and hence homozygous *Vbr* females cannot be produced. Heterozygous females were therefore used in the test-crosses but in all other respects these were carried out in an identical manner to those with *Ta*. The *Vbr* stock was maintained by repeated backcrosses to wild-type males which were of the same mixed genetic background as the *Ta* stock.

Table 2. *Expectated results of* Ta *and* Vbr *test-crosses should these loci be affected by the low and high 'states' of the controlling element*

'State' of X^T (= line of tested male)	Daughters			
	Genotype	Expected phenotype	Expected score	Expression of mutant
Low	Dp *Ta*$^+$/-*Ta*	More vibrissae	Higher	Low
High	Dp *Ta*$^+$/-*Ta*	Fewer vibrissae	Lower	High
Low	Dp *Vbr*$^+$/-*Vbr*	Less *Vbr*-variegation	Lower	Low
High	Dp *Vbr*$^+$/-*Vbr*	More *Vbr*-variegation	Higher	High

The level of expression of *Vbr* in the test-cross female progeny was estimated in a similar manner to that used in assessing the level of *c*-variegation in the translocation lines (Cattanach & Isaacson, 1967); the amount of white areas in the coats of individual females was estimated to the nearest 5 % and without knowledge of the identity of the animals. The scoring procedures were carried out when the animals were approximately 3 weeks old and classifications were made on as large groups as possible at any one time. This type of approach was found to reduce the errors liable to occur with this admittedly subjective scoring method. The level of *Vbr* was only estimated on the dorsal regions of the body for on the *agouti* background the paler belly hair often made the *Vbr* and *Vbr*$^+$ areas difficult to distinguish.

The translocation lines are carried on a *non-agouti, albino* coat colour background and hence the *c*-variegation is seen on a uniform black background. The *Ta* and *Vbr* stocks are carried on an otherwise wild-type background. The F_1 of each test-cross thus show only the *Ta* and *Vbr* phenotypes on the wild-type background and variegation due to the translocation is not seen.

Table 2 summarizes the type of result that might be expected in the *Ta* and *Vbr* test-crosses should those loci be affected by the different 'states' of the controlling element in the same manner as the rearranged *c* locus.

3. RESULTS

Since the expression of *Ta* is subject to modification by the genetic background, it is necessary to demonstrate that any differences observed between any two sets of crosses are not due to this factor. For this reason we have carried out the *Ta* test-crosses twice, first using the partially inbred Dp males and then the outcross Dp males. In addition, we have chosen to present the data in such a way that the results obtained with individual males can be seen. Two controls for any genetic

Table 3. *The influence of the* L *and* H *line* X^T *chromosomes on the expression of* Ta, *the* X^T *introduced through partially inbred Dp males*

Tested male			Progeny: Vibrissa scores			
			Ta/+ ♀♀		*Ta.* ♂♂	
Line	Identity no.	Progeny test *c*-variegation (%)*	No.	Score	No.	Score
L_2	5.2*d*	46·35 ± 2·90	12	16·50 ± 0·28	21	7·00 ± 0·34
	13.2*e*	47·27 ± 2·43	14	16·21 ± 0·38	31	6·61 ± 0·23
	14.1*i*	46·60 ± 2·44	21	16·85 ± 0·12	23	8·13 ± 0·36
	14.2*c*	46·25	18	17·00 ± 0·18	18	8·05 ± 0·47
	26.5*e*	51·54 ± 2·40	19	16·52 ± 0·17	24	7·33 ± 0·25
	36.3*i*	—	5	17·00	9	7·22
	Total	47·15 ± 1·24	89	16·67 ± 0·10†	126	7·34 ± 0·15‡
H_2	5.4*j*	55·67 ± 2·92	7	15·14	6	6·66
	8.4*j*	—	10	16·10 ± 0·23	14	7·86 ± 0·40
	16.1*d*	57·27 ± 1·89	6	15·00	6	7·5
	16.1*e*	62·50 ± 2·97	25	15·28 ± 0·38	24	7·25 ± 0·27
	16.3*i*	57·67 ± 2·28	11	15·18 ± 0·52	12	7·83 ± 0·66
	16.3*j*	59.28	2	14·50	4	9·50
	18.2*c*	57·25 ± 2·47	18	16·11 ± 0·29	26	6·73 ± 0·27
	33.1*d*	57·14	5	15·20	7	6·28
	Total	58·21 ± 1·01	84	15·49 ± 0·15†	99	7·27 ± 0·15‡

The progeny test scores were obtained in crosses with *c* females, the vibrissa scores in crosses with *Ta*/*Ta* females.

* The mean estimated levels of *c*-variegation in daughters of the Dp males in crosses with JU females; the 'true' levels are approximately 10–15 % lower.

† $t_{172} = 6{\cdot}72$; $P < 0{\cdot}001$.

‡ $t_{244} = 0{\cdot}33$; $P > 0{\cdot}7$.

background differences between the *L* or *LX* and *H* or *HX* crosses are available: (1) the scores of the *Ta* males, being further removed from the canalization zones than those of the *Ta*/+ females, should be more sensitive to genetic or environmental differences (Frazer & Kindred, 1960) and these should best demonstrate any such differences between the crosses; and (2) the data obtained from the tests of the chromosomally normal males should differ if any differences observed in the Dp male crosses are attributable to genetic background influences.

The results of the *Ta* test-crosses are presented in Tables 3 and 4 and in Table 3 the progeny-test scores of the *L* and *H* Dp males are also given. These *estimated*

levels of *c*-variegation (see legend to Table 3) are typical of those obtained in all progeny-tests, including those with outcross Dp males; the *LX* and *HX* animals employed in the test-crosses have not yet been progeny-tested. From both tables it can be seen that the mean vibrissa scores of the *L/LX Ta/+* females are higher than those of the equivalent *H/HX* females. The differences are statistically

Table 4. *The influence of the* L *and* H *line* X^T *chromosomes on the expression of* Ta, *the* X^T *introduced through outcross Dp males*

Tested male			Vibrissa score			
			Ta/+ ♀♀		*Ta* ♂♂	
Line	Identity no.	Genotype	No.	Score	No.	Score
L_4X	1.3*i*		20	17·25 ± 0·34	35	7·94 ± 0·25
	1.3*j*		17	16·53 ± 0·47	21	6·95 ± 0·25
	4.4*h*	Dp	20	16·50 ± 0·15	15	7·80 ± 0·44
	4.4*i*		18	16·78 ± 0·19	28	7·36 ± 0·36
	9.2*e*		12	17·08 ± 0·31	22	8·77 ± 0·32
	Total	Dp	87	16·82 ± 0·14*	121	7·77 ± 0·15†
	3.4*e*		35	17·11 ± 0·19	36	7·61 ± 0·23
	10.3*f*	Normal	28	17·46 ± 0·14	38	7·71 ± 0·26
	11.3*c*		31	17·22 ± 0·15	25	7·56 ± 0·29
	Total	Normal	94	17·26 ± 0·10‡	99	7·64 ± 0·15§
H_4X	1.1*g*		25	15·32 ± 0·38	26	7·23 ± 0·28
	5.1*f*		17	15·59 ± 0·51	20	8·65 ± 0·39
	6.2*d*	Dp	9	14·88	8	6·87
	6.2*e*		27	15·22 ± 0·29	49	7·29 ± 0·21
	24.1*f*		21	16·00 ± 0·30	26	7·27 ± 0·29
	Total	Dp	99	15·44 ± 0·18*	129	7·46 ± 0·14†
	2.3*b*		17	16·82 ± 0·29	28	8·03 ± 0·32
	5.1*h*	Normal	22	16·54 ± 0·16	26	7·89 ± 0·28
	6.2*c*		19	17·42 ± 0·25	25	6·68 ± 0·35
	Total	Normal	58	16·91 ± 0·15‡	79	7·56 ± 0·19§

* $t_{185} = 6{\cdot}02$; $P < 0{\cdot}001$. ‡ $t_{151} = 1{\cdot}94$; $P > 0{\cdot}05$.
† $t_{249} = 1{\cdot}50$; $P > 0{\cdot}05$ § $t_{177} = 0{\cdot}33$; $P > 0{\cdot}05$

significant in both experiments and that they are not due to exceptional crosses in each experiment is shown by the clear tendency for the individual family groups to exhibit scores typical of the cross as a whole. By contrast the mean *Ta* male scores do not differ significantly in either experiment. The female differences cannot therefore be attributed to genetic background influences; the *X*-chromosomes inherited from the Dp males must be responsible. It would appear that the rearranged (X^T) *X*-chromosomes which bring about the lower level of *c*-variegation, i.e. more wild type, in the progeny-tests also bring about a lower expression of *Ta* (more wild type) in the Dp$Ta^+/-Ta$ females, i.e. the vibrissa scores more

closely approach the normal number of 19 (see tables 3, 4). The banding pattern in the coats of the *Ta*/+ females was also affected. Females possessing the X^T of the *L* line more often did not display any *Ta* markings; genetic and/or cytological tests were necessary to distinguish them from partroclinous *XO* females.

The lack of importance of the genetic background in the test-crosses is confirmed by the results of the *LX* and *HX* crosses in which the chromosomally normal males were tested. Since both groups of animals possessed the *X* of the inbred (JU), only differences due to the genetic backgrounds would be expected. As

Table 5. *The influence of the* L *and* H *line* X^T *chromosomes on the expression of* Vbr, *the* X^T *introduced through partially inbred Dp males*

Tested male		Progeny		
			Vbr-variegation (%)	
Line	Identity no.	Progeny test *c*-variegation (%)*	No.	Score
L_2	5.2*d*	46·35 ± 2·90	15	42·66 ± 3·15
	14.1*i*	46·60 ± 2·44	18	46·66 ± 3·54
	26.5*e*	51·54 ± 2·40	7	43·57
	Total	47·21 ± 1·53	40	44·63 ± 2·13†
H_2	16.1*e*	62·50 ± 2·97	13	55·38 ± 5·35
	18.2*c*	57·25 ± 2·47	13	62·69 ± 3·68
	Total	59·88 ± 1·58	26	59·04 ± 1·03†

The progeny test scores were obtained in crosses with *c* females, the vibrissa scores in crosses with *Ta*/*Ta* females.

* The mean estimated levels of *c*-variegation in daughters of Dp males in crosses with JU females; the 'true' levels are approximately 10–15 % lower.

† $t_{65} = 6{\cdot}09$; $P < 0{\cdot}001$.

shown in Table 4, no differences in the *Ta* male vibrissa scores were detected and, although the mean *LX Ta*/+ female score tended to be higher than that of the *HX* females, the difference was not statistically significant. It should be noted that on the basis of the *Ta*/+ scores the JU *X*-chromosome is effectively indistinguishable from the X^T of the *L* lines.

The results of the *Vbr* test-crosses are presented in the same manner as those of the *Ta* test-crosses in Tables 5 and 6. It can be seen that essentially the same kind of response was obtained. The X^T which brings about a lower level of *c*-variegation also brings about a lower level of *Vbr*-variegation (more wild type) in the coats of the Dp $Vbr^+/-Vbr$ heterozgotes (see Table 5). Although there was no way of determining a *Vbr* male score to serve as a check on any genetic background influences, the test-crosses using the chromosomally normal *LX* and *HX* males again confirmed that such an influence is not responsible for the female differences in the Dp crosses. Again it should be noted that as in the *Ta* test-crosses, the JU *X*-chromosome is effectively indistinguishable from the X^T of the *L* line.

Table 6. *The influence of the* L *and* H *line* X^T *chromosomes on the expression of* Vbr, *the* X^T *introduced through outcross Dp males*

Tested male		Progeny		
			Vbr-variegation (%)	
Line	Identity no.	Genotype	No.	Score
L_4X	3.2*e*	Dp	14	38·93 ± 3·68
	3.5*g*		12	41·67 ± 4·45
	7.1*h*		18	41·94 ± 3·38
	13.1*d*		16	44·69 ± 4·24
	Total	Dp	60	41·92 ± 1·91*
	3.4*e*	Normal	25	40·00 ± 3·26
	11.3*c*		20	44·25 ± 3·67
	Total	Normal	45	41·89 ± 2·43†
H_4X	6.1*g*	Dp	9	53·33 ± 5·00
	6.2*e*		15	49·67 ± 3·79
	11.1*j*		15	53·00 ± 3·95
	3.2*j*		5	52·00
	24.1*f*		25	53·60 ± 3·52
	Total	Dp	69	52·50 ± 1·96*
	5.1*h*	Normal	15	42·33 ± 4·00
	2.3*b*		28	44·46 ± 2·15
	Total	Normal	43	43·72 ± 1·96†

* $t_{123} = 3{\cdot}86$; $P < 0{\cdot}001$. † $t_{87} = 0{\cdot}59$; $P > 0{\cdot}5$.

4. DISCUSSION

The results of the test-crosses clearly indicate that the levels of expression of *Ta* and *Vbr* in the heterozygous female is influenced by some property of the *X*-chromosome itself. Each Dp male possessing the *L* line X^T produced *Ta*/+ (Dp*Ta*$^+$/−*Ta*) daughters with higher mean vibrissa scores and *Vbr*/+ (Dp *Vbr*$^+$/− *Vbr*) daughters with lower amounts of *Vbr* in their coats than the equivalent daughters of Dp males possessing the *H* line X^T. Thus, the shift in the level of expression of all three characters (*c*, *Ta*, *Vbr*) attributable to one X^T relative to the other always lay in the same direction (see Tables 3–5). Since the possibility is so remote that there could be independent *X*-linked modifiers of each locus which only influence the heteroyzgous phenotype and which always shift the phenotype of each character in the same direction, there can be little doubt that only a single modifiying system is operating, i.e. the controlling element system detected with the use of the translocation.

That the controlling element system is not restricted to the rearranged *X*-chromosomes is demonstrated by the results of test-crosses using chromosomally normal animals. As observed with the X^T chromosome, the influence of the JU *X* on the expression of *Vbr* paralled its influence on *Ta*. In addition, the JU *X* could

not be effectively distinguished in individual test-crosses from the *L* line X^T. This observation becomes more meaningful when some information from an accompanying experiment is introduced. It has been found that changes in the 'state' of the *H* line X^T controlling element can occur on the JU inbred background, i.e. when heterozygous with the *X* of the inbred, but that this does not appear to be true of the *L* line X^T (Cattanach, Perez & Pollard, in press). When the results of both experiments are considered together it may be concluded that the JU *X*-chromosome possesses the *L* line X^T 'state' of the controlling element and that the observed changes in 'state' of the *H* line X^T element most likely occurs as a result of crossing over between the X^T and the normal *X*-chromosome. It should be added that when such changes are detected, the changed *H* line X^T chromosomes cannot be distinguished from the JU *X* or *L* line X^T in either the *Ta* or *Vbr* test-crosses; the level of expression of all three characters changed together. Since it is known that the *L* line X^T can also change to a form indistinguishable from the *H* line X^T when heterozygous with other normal *X*-chromosomes (Cattanach & Isaacson, 1967), indirect evidence for the existence of normal *X*-chromosomes possessing the *H* line X^T 'state' of the controlling element exists. Such chromosomes should be recoverable from the *H* line by crossing over.

Further evidence that there is a controlling element system in normal *X*-chromosomes which can modify the heterozygous phenotypes of sex-linked genes can be derived from three independent sets of data:

(1) Falconer & Isaacson (1969) have found that the degree of expression of *Brindled*, another allele at the *Mottled* locus, can be modified in the heterozygote and that this is controlled by some property of the *X*-chromosome itself. Their experiment was specifically designed to test the possibility of non-random *X*-inactivation and their results conclusively disproved this to be a possible mechanism. The data could also rule out cell selection as the the responsible mechanism.

(2) B. M. Kindred (personal communication) on crossing several lines of *Ta* mice selected over several generations for high and low secondary vibrissa numbers has found that there are *X*-linked modifiers of *Ta* which are closely linked to the *Ta* locus and which only modify the allele that has been under selection.

(3) M. F. Lyon (personal communication) has created a line of mice in which *Gyro*, an *X*-linked gene located at the extreme end of the linkage group, exhibits a high penetrance in the heterozygous female, and she has found that 'high *Gyro*' will disappear and arise as a result of crossing over with other *X*-chromosomes.

All three sets of data suggest that there are *X*-linked modifiers of *X*-linked loci and that these only operate upon the allele located in the same chromosome. The parallelism of the data with those presented here and with those of our earlier publications (Cattanach & Isaacson, 1965, 1967) strongly suggests that a single mechanism is responsible. Kindred's observation that the modifiers of *Ta* are closely linked to the *Ta* locus is particularly intriguing since the controlling element is known to be closely linked to the translocation break-point (Cattanach & Isaacson, 1967), and in more recent estimates (Cattanach, Perez & Pollard,

in press) it has been shown to be located at about the same distance from the break-point as is *Ta*.

Since the results of the present experiments indicate that the controlling element system can modify the heterozygous expression of *X*-chromosomal genes it would seem most likely that the effect is brought about by the same or by a related mechanism to that controlling the *c*-variegation. The system is considered to operate by determining the frequency with which the rearranged autosomal loci are inactivated in those cells in which the X^T is inactivated, i.e. the different 'states' of the element influence the inactivating properties of the *X* such that the position effect variegation is modified (Cattanach & Isaacson, 1965, 1967). A seemingly analogous situation has recently been described in *Drosophila*. Spofford (1967) has shown that an allele at a locus closely linked to the duplication, *Dp* (1 ; 3) $w^{m264.58a}$, suppresses position effect variegation for *w*, and Cohen (1962) has shown that different 'states' of the duplication influence the variegation of neighbouring rearranged loci in the same manner as *w*. Presumably the modifier is altering the inactivating properties of the associated heterochromatin. In the present system, it might be postulated that the controlling element is responsible for the inactivation of the *X* and that the different 'states' differ in degree of effectiveness with regard to this property.

Before developing this concept it is necessary to demonstrate that the modification of the heterozygous phenotypes cannot be attributed to other possible mechanisms, i.e. non-random *X*-inactivation or selection operating upon the two cell populations that result from *X*-inactivation. The data of Falconer & Isaacson (1969) undoubtedly provide the best evidence that these mechanisms are not operating with normal *X*-chromosomes, but the evidence obtained from the translocation experiments is also quite convincing (Cattanach & Isaacson, 1965, 1967). (1) It has been established that the level of *c*-variegation is solely dependent upon the X^T; substitution of one normal *X* for another does not influence the variegated phenotype. This is also evident in the test-crosses where the parallelism in the results indicates the X^T is responsible for the levels of expression of *Ta* and *Vbr*. (2) The mean level of *c*-variegation never exceeds 50 %, i.e. there is always an excess of wild type. The 50 % maximum is important, for it suggests that the responsible mechanism is only operating upon one *X*—the X^T.

A related consideration is that the hemizygous Dp genotype has a low viability at the organism level (Cattanach, 1961) and it is therefore unlikely that at the cell level the Dp genotype would have any selective advantage over the normal. Again, the parallelism of the results obtained with *Ta* and *Vbr* indicates that the mechanism is not limited to a single cell system; the effect is seen in two and possibly three different cell populations. Further, the apparent advantage of the X^T chromosomes over the normal in the translocation lines and test-crosses are duplicated by the apparent advantage of the same normal *X*-chromosome when subjected to the same test-crosses. When all the data are considered together, it would seem most unlikely that the differences between the *H* line X^T and *L* line X^T or JU *X*, as observed in the progeny-tests and test-crosses, could be the consequence of non-

random *X*-inactivation or selective mechanisms operating at the cellular level. Rather it seems that the levels of *c*-variegation characteristic of each X^T are dependent upon the inactivating properties of the *X* and that these properties relate to the level of inactivation of the *X* itself. It may thus be proposed that *X*-chromosome inactivation can be 'incomplete', the level of inactivation or the frequency of cells in which inactivation is incomplete being dependent upon the 'state' of the controlling element located in the *X*.

Incomplete inactivation of the mammalian *X*-chromosome has long been considered to be a possibility primarily because, as a mechanism of dosage compensation, *X*-inactivation appears to be an imperfect process, e.g. in man aneuploidy of the *X* is associated with abnormal phenotypes. The line of thought has been that there might be a region of the *X*, both in man (Ferguson-Smith *et al.* 1964; Ferguson-Smith, 1965) and in the mouse (Russell, 1963), that is free of the inactivation process, or that some loci may not be inactivated (Beutler, 1964). The results of the present experiments suggest quite a different level of incomplete inactivation, for it pertains to loci that are clearly subject to inactivation.

It has previously been proposed (Cattanach & Isaacson, 1967) that the mouse *X*-chromosome controlling the element can be equated to the postulated *X*-inactivation centre (Grumbach, 1964; Lyon, 1964; Russell, 1964) and the results of the present experiments strongly support this concept. If this is true, it would seem unlikely that there is a spread of inactivation from the inactivation centre along the *X* in the same manner as across regions of adjoining autosome as suggested by Russell (1964). The controlling element is known to be centrally located in the linkage group (Cattanach & Isaacson, 1967; Cattanach, Perez & Pollard, in press), and although the two *X*-linked genes studied in the present experiment are also located in this region our data suggest that they need not be completely inactivated. Moreover, were there a spread of inactivation along the *X*, more remote loci might be expected to be free of the inactivation process, and yet, for the one gene that can be tested, this has proven to be untrue (Lyon, 1966).

An alternative possibility is that the spreading effect observed with the rearranged autosomal genes only indicates some change in the inactivation of the *X*-chromosome as a whole. Observations on the nature of the position effect variegation (Cattanach, unpublished) suggest this variegation is attributable to the reversal of inactivation of the rearranged autosomal loci in some melanocytes migrating laterally down the sides of the body (Rawles, 1948; Mintz, 1967). Such pigmented clones of cells can be seen as spots or 'half-bands' in an otherwise non-pigmented (*albino*) background. By analogy with this aspect of the position effect variegation it may be proposed that in certain clones of cells *X*-chromosomal loci may be reactivated or, at least, show some degree of activity. Evidence of such a reversal of inactivated chromosomes during the development has been obtained in some species of mealy bugs (Nur, 1967). In these species, loss or damage to the apparently inactive, heterochromatic chromosomes results in developmental abnormalities as does aneuploidy of the *X*-chromosome in mammals, notably in man.

In concluding, it should be pointed out that the stress laid on the involvement of

only one of the two X-chromosones, the X^T, in the control of the position effect variegation need not apply to variegation for the X-linked genes. These loci are present on both chromosomes and, depending on the 'state' of the controlling element carried on each chromosome, one or both alleles may be completely or incompletely inactivated. Depending on the dominance relations of the gene under study a range of phenotypes from a clear-cut mosaic to a diffuse mosaic could be expected.

SUMMARY

The mouse X-chromosome controlling elements, detected by their influence on the position effect variegation caused by the X-autosome translocation $T(1;X)Ct$, have been found to modify the heterozygous phenotypes of two X-linked genes. It is proposed that X-inactivation can be incomplete, the level of inactivation or the frequency of cells in which inactivation is incomplete being dependent upon the 'state' of the controlling element located in the X. The data suggest that this is a consequence of a reversal, or partial reversal, of inactivation of the X as a whole in some cells rather than a vairable spread of inactivation along the length of the X.

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