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Further evidence for the importance of parental source of the Xce allele in X chromosome inactivation

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Summary

Using mice that were mosaics for both *Xce* and phosphoglycerate kinase (*Pgk-1*) alleles, we present further evidence that the parental source of the X chromosome may affect the probability of that X chromosome remaining active. The reciprocal cross differences in PGK-1 activity described here are intermediate between those published previously for other alleles of *Xce*.

1. Introduction

With few exceptions (Migeon *et al.* 1982; Shapiro *et al.* 1979; Fialkow *et al.* 1970), most gene loci of one of the two X chromosomes are inactivated in individual embryonic cells of female eutherian mammals shortly after implantation. A compensation mechanism thereby exists such that females and males have the same dosage of X-linked genes. X-inactivation is stably inherited, that is, once the process is initiated, the inactivated state of the X chromosome is maintained through successive cell divisions in virtually all cases (Migeon, 1972; Kahan & DeMars, 1975). In general, inactivation of the maternally derived (X^M) or the paternally derived (X^P) X chromosome is random in the embryo (Lyon, 1962; Evans *et al.* 1965; Mukherjee *et al.* 1970), while in extraembryonic membranes, preferential X^P-inactivation prevails (Takagi & Sasaki, 1975; West *et al.* 1977; Frels *et al.* 1979; Papaioannou & West, 1981).

Evidence for the existence of a locus that influences the probability of X-inactivation comes from studies involving the mouse X-chromosome-controlling element (*Xce*) (Cattanach & Isaacson, 1967; Cattanach *et al.* 1969, 1970). Three alleles of this locus are recognized – Xce^a, Xce^b and Xce^c (Johnston & Cattanach, 1981; Cattanach & Papworth, 1981). In *Xce* heterozygotes, cells with an active Xce^b chromosome are statistically more numerous than those with an active Xce^a and cells with an active Xce^c chromosome are more numerous than those with an active Xce^b (i.e. $Xce^c > Xce^b > Xce^a$). This phenomenon is considered to be a result of non-random X-inactivation rather than cell selection.

The *Xce* locus is closely linked to the phosphoglycerate kinase-1 (*Pgk-1*) locus on the mouse X chromosome (Green, 1981; Krietsch *et al.* 1986). Recombinants between *Xce* and *Pgk-1* are rare and since Xce^c segregates with $Pgk\text{-}1^a$ and other *Xce* alleles with $Pgk\text{-}1^b$, analysis of the relative proportions of PGK-1A and PGK-1B alloenzymes in a population of cells will reveal the effect of *Xce* on X-inactivation. Non-random expression was found when Xce^c was heterozygous with Xce^b (% PGK-1A > % PGK-1B) and more extreme non-random expression was found in the Xce^c/Xce^a combination (Johnston & Cattanach, 1981). Few strains of mice have been classified with respect to *Xce*.

Parental effects on X-inactivation were reported for adult tissue in Xce^b/Xce^c heterozygotes by Forrester & Ansell (1985). Sixty per cent PGK-1A expression was found when the Xce^b was paternally derived, while X-inactivation was equally balanced when the Xce^b was maternally derived. No such parental effects were found in Xce^c/Xce^a combinations.

We now report the results of reciprocal crosses between AKR/J ($Pgk\text{-}1^b$) $Xce^?$) and $Pgk\text{-}1^aXce^c$ mice. These data provide further evidence for parental effects on X-chromsome inactivation.

2. Materials and methods

(i) *Mice*

The variant $Pgk\text{-}1^a$ allele was discovered in Danish feral mice (Nielsen & Chapman, 1977) and backcrossed for eight generations onto the standard laboratory strain C3H/HeHa ($Pgk\text{-}1^b$) by Drs J. D.

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West and V. M. Chapman, RPMI, Buffalo, NY). The C3H/HeHa(*Pgk-1*^{a}) mice, originally obtained from Dr West, were maintained in this laboratory by sib-mating. CBA(*Pgk-1*^{a}) mice were produced in this laboratory by backcrossing C3H(*Pgk-1*^{a}) mice onto a CBA/Ca(*Pgk-1*^{b}) background for 20 generations. *Pgk-1* and *Xce* are tightly linked on the X chromosome and no recombination was observed between these loci in our backcrossing programme (Forrester & Ansell, 1985). This observation was confirmed by Krietsch *et al.* (1986), who similarly observed no recombinants after 15 generations of backcrossing. CBA/Ca(*Pgk-1*^{b}) males were subsequently mated to CBA(*Pgk-1*^{a}) females to produce CBA(*Pgk-1*^{ab}) heterozygous females. AKR/J mice were originally obtained from Olac Ltd, Bicester, UK and maintained here by sib-mating.

(ii) *Preparation of samples*

One drop of blood (approximately 20 μl) was removed from the retro-orbital sinus of mice (2 months of age) and mixed with 100 μl of sample buffer (50 mM triethanolamine HCl, pH 7.6, containing 0.3 mg/ml dithioerythritol, 0.5 mg/ml bovine serum albumin and 2 mg/ml digitonin). Electrophoresis and quantification of PGK-1 alloenzymes by the MTT method are described elsewhere (Ansell & Micklem, 1986). As an estimation of technical variation, a single artificial sample composed of 50% PGK-1A spleen cells and 50% PGK-1B spleen cells was assayed ten times on each of eight individual cellulose acetate membranes and gave a within-membrane standard error of 0.2–0.8%. The between-membrane standard error was 0.6%.

3. Results and discussion

A significant difference in mean % PGK-1A expression was observed between reciprocal crosses with AKR/J parents (Table 1). Thus, although the *Pgk* − 1^a, *Xce*^{c}-bearing X chromosome was less likely overall to be inactivated, it was more likely to be inactivated when it was paternally derived than when it was maternally derived.

Since significant reciprocal cross differences were noted between crosses A and B, and C and D and the mean %PGK-1A in these hybrids differed significantly from that for the known *Xce*^{a}/*Xce*^{c} heterozygotes (strain, E, $P < 0{\cdot}05$), this suggested that the AKR/J strain did not carry the 'a' allele for *Xce* ascribed to the normal CBA and C3H mouse strains.

Forrester & Ansell (1985) described reciprocal cross difference in X-inactivation frequencies in heterozygotes derived from C57BL and C3H/HeHa-Pgk-1^a parental strains carrying the 'b' and 'c' alleles of *Xce* respectively. The direction of the reciprocal cross differences in AKR/J heterozygotes described in Table 1 agrees with that of Forrester & Ansell (1985), although actual percentages differ: they found 52% PGK-1A (data combined from two groups) when *Xce*^{c} was of paternal origin compared to 60% in crosses A and C above ($P < 0{\cdot}001$) and 60% PGK-1A (data combined from three groups) when *Xce*^{c} was of maternal origin compared to 65% in cross B ($P < 0{\cdot}001$) and D ($P < 0{\cdot}01$). These data suggest the existence of a fourth allele for *Xce* carried by the AKR/J mouse strain, subject to parental influences on its expression, but distinct from the 'b' allele. However, background effects of the AKR/J genotype on the expression of *Xce* and/or *Pgk-1* cannot be excluded.

Variations in the proportions of PGK-1 alloenzymes in tissues are unlikely to be due to differences in the expression of enzyme activity since in mice that are homozygous for the *Xce*^{c} but heterozygous for *Pgk*-1 alleles, the proportions of the two alloenzymes in adult tissues do not deviate significantly from the expected 50:50 ratio (Krietsch *et al.* 1986). Data (not shown) also indicate no selective effect of any particular allele with age in any of the subpopulations of peripheral blood or lymphoid organs analysed. Thus, the present data from AKR (*Pgk-1*^{ab}) heterozygotes confirm that the parental provenance of *Xce* alleles is important in determining whether maternal or paternal X chromosomes remain active.

The influence of the parental source of the X

Table 1.

	Male parent			Female parent			Mean	
Hybrid	Strain	*Pgk*-1	Xce	Strain	*Pgk-1*	*Xce*	% PGK-1A + S.E.	*N*
A	C3H	a	c	AKR/J	b	?	60 + 0·7	149
B	AKR/J	B	?	C3H	a	c	65 + 0·8 ($P < 0{\cdot}001$)	134
C	CBA	a	c	AKR/J	b	?	60 + 1·3	27
D	AKR/J	b	?	CBA	a	c	65 + 1·6 ($P < 0{\cdot}02$)	28
E	CBA	b	a	CBA	a	c	69 + 1·7	30
*[F	CBA	a	c	CBA	b	a	72 + 1·4	44]

* Taken from Forrester & Ansell (1985).

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chromosome and its interaction with *Xce* alleles in determining the frequency with which X^M or X^P remains active has been discussed by several authors, but the direction of these effects remains controversial. Some authors have found no reciprocal cross differences (Johnston & Cattanach, 1981) while others demonstrate a 'paternal' effect, i.e. X^P having a higher probability of remaining active (Cattanach, 1975; Falconer *et al.* 1982). In selection experiments with the X-linked brindled alleles Falconer *et al.* (1982) and Cattanach & Papworth (1981) found a positive correlation between the expression of brindled in mothers and daughters. However, this 'maternal' effect was attributed to a physiological difference rather than the parental origin of the chromosome. In extraembyonic membranes parental effects on X chromosome inactivation are most striking, X^M remaining active almost to the exclusion of X^P (Takagi & Sasaki, 1975; West *et al.* 1977; Frels *et al.* 1979; Papaioannou & West, 1981). Parental influences on X chromosome expression are influenced by the expression of different *Xce* alleles (Cattanach, 1975; Rastan & Cattanach, 1983; Bucher & Krietsch, 1988). Rastan & Cattanach (1983), for example, have demonstrated that 'strong' alleles at the *Xce* locus can override the maternal effect even in extraembryonic membranes; i.e. when X^P carries the Xce^c allele, these tissues do not exclusively express X^M. Similarly, no parental effects on PGK-1 expression were seen in reciprocal crosses carrying extreme alleles at the *Xce* locus (Forrester & Ansell, 1985) although Bucher & Krietsch (1988) have detected parental effects in those situations. Background genetic effects may influence the direction and degree of parental effects, although in different strain combinations the data presented above supports the conclusion of Forrester & Ansell (1985) that maternal inheritance of *Xce* alleles mitigates against X^M being inactivated.

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