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# Parental influences on *X* chromosome expression

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SUMMARY

Using mice that were mosaics for both *Xce* and phosphoglycerate kinase (*Pgk*-1) alleles, we have established that the parental source of the *Xce* gene may affect the probability with which the *X* chromosome carrying it will remain active. This effect was seen in one allelic combination of *Xce* but not in another. The relationship between these effects and other phenomena of maternal 'imprinting' is discussed.

## 1. INTRODUCTION

In female eutherian mammals, *X* chromosome inactivation results in individual somatic cells expressing either the maternally derived *X* chromosome ($X^M$) or the paternally derived *X* chromosome ($X^P$) (Lyon, 1961). In some circumstances genetic (Cattanach & Isaacson, 1965) and/or parental effects (Takagi & Sasaki, 1975; West, Frels & Chapman, 1977) modify the *X* inactivation process, resulting in $X^M$ and $X^P$ having an unequal probability of becoming inactivated.

Work in the mouse has suggested that there is an inactivation centre, present on the *X* chromosome, designated the *X* chromosome controlling element (*Xce*) (reviewed by Cattanach, 1975). Three alleles of the *Xce* locus have been described and they affect the probability that a particular *X* chromosome will become inactivated. Analyses of females heterozygous at the *Xce* locus (and also at other *X* linked marker loci) have shown that an *X* chromosome carrying the $Xce^a$ allele is more likely to become inactivated than an *X* chromosome bearing the $Xce^b$ allele. Similarly an $Xce^b$ *X* chromosome has a higher probability of inactivation than an *X* chromosome bearing a third allele, $Xce^c$.

The existence of parental factors which influence the *X* inactivation process is most obvious in studies on the extraembryonic membranes (trophectoderm and primitive endoderm) of the mouse embryo, where the paternally derived *X* chromosome ($X^P$) is preferentially inactivated (Takagi & Sasaki, 1975; West *et al.* 1977). Embryo transfer and oocyte transplantation experiments have shown that the maternal reproductive tract does not exert a selection pressure in favour of cells expressing $X^M$ (Frels & Chapman, 1980; Papaioannou & West, 1981). It is thought that the differential expression is due to an intrinsic difference between $X^M$ and $X^P$, imprinted before the *X* inactivation process, although the molecular mechanism for this imprinting remains unknown.

Parental effects on *X* chromosome inactivation in somatic tissues have been

studied by comparing the phenotypes of females, heterozygous for *X*-linked gene products, derived from reciprocal crosses. Some studies indicate a 'paternal' effect i.e. $X^P$ has a higher probability of remaining active than $X^M$ (Falconer, Isaacson & Gauld, 1982), while others show no significant reciprocal cross differences (Johnston & Cattanach, 1981). In selection experiments with the *X*-linked gene brindled (*Mo*$^{br}$) (Falconer *et al.* 1982) and viable brindled (*Mo*$^{vbr}$) (Cattanach & Papworth, 1981), a positive correlation was observed between the expression of brindled in mothers and daughters. However, this 'maternal' effect was attributed to abnormal copper transport in the heterozygous mothers rather than a chromosomal effect. Using alleles of phosphoglycerate kinase as markers for *Xce* we have found no evidence of a parental source effect in females that are heterozygous for the *a* and *c* alleles of *Xce*. However, we show that in females that were heterozygous for the *b* and *c* alleles of *Xce* the *X* chromosome carrying the *Xce*$^c$ alleles has a higher probability of remaining active if it is maternally derived than if it is paternally derived.

## 2. MATERIALS AND METHODS

### (i) *Mice*

The strains C3H/HeHa-*Pgk-1*$^a$*Xce*$^c$ (backcross generation 9) and CBA/Ca-*Pgk-1*$^a$*Xce*$^c$ (backcross generation 16) were used as the source of the *X* chromosome carrying the *Pgk-1*$^a$ and *Xce*$^c$ alleles; the C57BL/6J-*bg*$^J$ strain for the *X* chromosome carrying the *Pgk-1*$^b$ and *Xce*$^b$ alleles and the CBA/Ca for the *Pgk-1*$^b$ and *Xce*$^a$ alleles. *Pgk-1* and *Xce* are closely linked (Cattanach, Perez & Pollard, 1970; Franke & Taggart, 1980) and any recombination between these two loci would be expected to be infrequent. An extensive backcrossing programme in our laboratory supports this expectation.

### (ii) *Preparation of samples*

One drop of blood taken from the retro-orbital sinus of 3–4 week-old females was mixed with 100 $\mu$l 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 the two PGK-1 alloenzymes were carried out as described elsewhere (Woodruff *et al.* 1982; Ansell & Micklem, 1984). Repeats of a single sample within and between gels give an overall standard deviation of approximately 2·1 %. A linear relationship is observed between different artificial mixtures of PGK-1 alloenzymes over the range from 20 to 80 % PGK-1A.

## 3. RESULTS

Table 1 summarizes the results of phenotypic analyses of heterozygous females derived from 5 different crosses. Crosses 1 and 2 were reciprocal crosses between C3H/HeHa-*Pgk-1*$^a$ *Xce*$^c$ and C57BL/6J-*bg*$^J$ strains. In cross 1 where the *Xce*$^c$ *Pgk-1*$^a$ bearing *X* chromosome was maternally derived, the mean proportion of PGK-1A alloenzyme present in the blood of heterozygous progeny was 61 %. When the *Xce*$^c$*Pgk-1*$^a$ bearing *X* chromosome was paternally derived (cross 2) the mean

proportion of PGK-1A present was 54 %. The difference between these reciprocal crosses was statistically significant ($P < 0{\cdot}01$).

The Fl males (*Xce$^c$Pgk-1$^a$/ Y*) produced from cross 1 were then backcrossed onto the C57BL/6J-*bg$^J$* strain (cross 3). In this case, the *Xce$^c$Pgk-1$^a$ X* chromosome was paternally derived and the proportion of PGK-1A present was 51 %. The F1 females (*Xce$^c$Pgk-1$^a$/Xce$^b$Pgk-1$^b$*) produced from cross 2 were also backcrossed onto the C57BL/6J-*bg$^J$* strain (cross 4). The proportion of PGK-1A in the tissues of heterozygous females produced in this case was 58 %. This was significantly different from the proportion in cross 3 ($P < 0{\cdot}01$). The heterozygous females produced from cross 3 were further backcrossed onto the C57BL/6J-*bg$^J$* strain (cross 5) and the proportion of PGK-1A in the heterozygous progeny of this cross was 60 %.

Table 1. *Percentage PGK-1A in mice heterozygous for the* b *and* c *alleles of* Xce

| Cross | *Xce* allele of parental *X* chromosome | | Mean percentage of PGK-1A in progeny ± S.E. | No. progeny analysed |
|---|---|---|---|---|
| | $X^M$ | $X^P$ | | |
| 1 | *c* | *b* | 61 ± 1·5 | 36 |
| 2 | *b* | *c* | 53 ± 1·1 | 36 |
| 3 | *b* | *c* | 51 ± 1·3 | 56 |
| 4 | *c* | *b* | 58 ± 1·8 | 32 |
| 5 | *c* | *b* | 60 ± 1·7 | 32 |

Analysis of variance showed that the progeny of crosses 1, 4 and 5 were significantly different from crosses 2 and 3 ($P < 0{\cdot}01$). In these crosses *Pgk-1$^a$* segregates with *Xce$^c$* and *Pgk-1$^b$* with *Xce$^b$*.

Table 2. *Percentage PGK-1A in mice heterozygous for the* a *and* c *alleles of* Xce

| *Xce* allele of parental *X* chromosome | | Mean percentage of PGK-1A in progeny ± S.E. | No. progeny analysed |
|---|---|---|---|
| $X^M$ | $X^P$ | | |
| *c* | *a* | 69 ± 1·5 | 27 |
| *a* | *c* | 72 ± 1·4 | 44 |

These data were derived from reciprocal crosses of sublines of CBA/Ca mice differing only at the region of the *Pgk-1* and *Xce* loci. There was no significant difference in the proportion of PGK-1A between the offspring of either cross. In these crosses *Pgk-1$^a$* segregates with *Xce$^c$* and *Pgk-1$^b$* with *Xce$^a$*.

There was no significant difference between the PGK-1 phenotypes of the heterozygous progeny of crosses 1, 4 and 5. In all these cases the *Xce$^c$Pgk-1$^a$*-bearing *X* chromosome was maternally derived and the proportion of PGK-1A present was 58–61 %. Also, there was no significant difference in the heterozygous phenotypes of the progeny produced from crosses 2 and 3. The *Xce$^c$Pgk-1$^a$ X* chromosome in these crosses was paternally derived and the proportion of PGK-1A present was 50–53 %.

A large series of reciprocal crosses between CBA/Ca (*Pgk-1$^b$ Xce$^a$*) and CBA/Ca-

*Pgk-1*$^a$*Xce*$^c$ mice were analysed (Table 2). In this case the 'strong' allelic combination of *Xce* (*Xce*$^a$/*Xce*$^c$) was involved. No reciprocal cross differences were observed in this series.

## DISCUSSION

In the extraembraembryonic membranes of the mouse, $X^M$ is preferentially expressed (Takagi & Sasaki, 1975) but the existence of parental effects are less well defined in the embryo proper and adult somatic tissues. $X^M$ and $X^P$ may have an equal probability of becoming inactivated (Johnston & Cattanach, 1981) or in some circumstances $X^P$ may be preferentially expressed (Falconer *et al.* 1982). Using PGK-1 alloenzyme expression in erythrocytes, we have found parental effects on $X$ chromosome expression using one allelic combination of *Xce* genes but no such effects with another. In the first case the parental source of the *Xce*$^c$ allele changed the probability of it being expressed in *Xce*$^b$/*Xce*$^c$ heterozygotes from approximately 0·6 to 0·5, when maternally or paternally derived respectively. However, in *Xce*$^a$/*Xce*$^c$ heterozygotes the parental source of *Xce*$^c$ did not affect the probability of its expression. Although erythrocytes are constantly being replenished the variance in the proportions of PGK-1 alloenzymes in the blood of individual mice, bled regularly for up to 1 year, is small (Micklem *et al.* 1984). Peripheral blood can therefore be regarded as having a stable phenotype and being representative of haematopoietic tissues. Analyses of a small number of thymus and bone marrow samples from offspring of crosses 1, 2, and 3 (data not shown) confirm this assumption. Since all tissues are derived from the same pool of $X$-inactivated cells (McMahon, Fosten & Monk, 1983), analyses of any tissue should show similar results to those described in the blood and other haematopoietic tissues. Preliminary data from kidney and brain samples tend to support this hypothesis.

It is probable that we are looking at an interaction between parental and genetic effects. Previous analyses of *Xce*$^b$/*Xce*$^c$ females would predict that (in the absence of other influences) an *Xce*$^c$*Pgk-1*$^a$ $X$ chromosome would have a higher probability (approx. 0·6) of remaining active than an *Xce*$^b$*Pgk-1*$^b$ $X$ chromosome (Johnston & Cattanach, 1981). We have found that when the *Xce*$^c$*Pgk-1*$^a$ $X$ is maternally derived, this prediction is borne out. However, when the *Xce*$^c$*Pgk*-1$^a$ $X$ is paternally derived the probability of that $X$ chromosome being expressed is reduced to approximately 0·5. The mechanism by which these two effects interact is unknown but could be envisaged as West (1982) proposed. The physiological modification of $X^M$ and/or $X^P$ would presumably include the *Xce* locus and the modification of one *Xce* allele could affect its interaction with the other in a heterozygous female. This could have either an additive or a complementary result. If for example the modification of the genome during oogenesis results in the *Xce*$^b$ allele on $X^M$ behaving in a similar way to an *Xce*$^c$ allele, the heterozygous female produced (if $X^P$ carries *Xce*$^c$) would behave as if it were homozygous at the *Xce* locus. In this hypothetical example $X^M$ and $X^P$ would have an equal probability of expression.

The preferential inactivation of $X^P$ in the earliest differentiated tissues of the embryo, i.e. the trophectoderm and primary endoderm, suggests that $X^M$ and $X^P$ are differentially marked before the $X$ inactivation process. When these lineages

differentiate this 'imprinted' difference between $X^M$ and $X^P$ is still present, resulting in $X^P$ being preferentially inactivated. Cell lineages that differentiate later express both $X^M$ and $X^P$, suggesting that this 'imprinting' is short-lived. The reciprocal cross difference observed in $Xce^b/Xce^c$ heterozygotes in our experiments could indicate that residual effects of the 'imprinting' process remain. However, Rastan & Cattanach (1983) have shown that 'strong' alleles at the *Xce* locus can override the maternal effect in the extraembryonic membranes. When $X^P$ carries the $Xce^c$ allele, the extraembryonic membranes do not exclusively express $X^M$. We did not see any parental effects on $X$ chromosome expression in the erythrocytes of mice, heterozygous for the 'strong' allelic combination ($Xce^a/Xce^c$). It is possible that this allelic combination is too extreme for the more subtle maternal influences to affect it. Alternatively, it is possible that other moderator genes are causing the reciprocal cross difference and the expression of these genes differs between different mouse strains. The reciprocal cross differences observed were between two different strains (C3H/HeHa-$Pgk\text{-}1^a$ $Xce^c$ and C57BL/6J-$bg^J$) whereas the crosses that did not show these differences were between mice which had been backcrossed onto the CBA/Ca strain for 16 generations and ostensibly differed only in the region of the *Pgk-1* and *Xce* loci. It is possible that the reciprocal cross differences are only seen between different strains or between strains with particular moderator genes being expressed. Backcrossing of the *Pgk-1* and *Xce* loci onto the C57BL/6J-$bg^J$ strain would perhaps resolve this question.

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