Genet. Res., Camb. (1981), **37**, *pp.* 183–197
With 2 text-figures
Printed in Great Britain

Relationship between the parental origin of the *X* chromosomes, embryonic cell lineage and *X* chromosome expression in mice

VIRGINIA E. PAPAIOANNOU AND JOHN D. WEST
Sir William Dunn School of Pathology, South Parks Road, Oxford OX1 3RE, U.K.

(*Received* 2 *October* 1980)

SUMMARY

The electrophoretic variants of the *X*-chromosome-linked enzyme phosphoglycerate kinase (PGK-1) have been used to investigate the randomness of *X* chromosome expression in the fetus and various extraembryonic membranes of the mouse conceptus. The amnion shows essentially random expression of the maternally derived *X* chromosome (X^m) and the paternally derived *X* chromosome (X^p). The parietal endoderm, however, shows exclusive or preferential expression of X^m. The results support the idea that the randomness of *X* chromosome expression is correlated with embryonic cell lineage such that X^m is preferentially (perhaps exclusively) expressed in derivatives of the primitive endoderm and trophectoderm but that X^m and X^p are randomly expressed in the derivatives of the primitive ectoderm.

Experiments involving ovary transplants, embryo transfers or crosses with heterozygous mothers confirm previous findings that X^m is preferentially expressed regardless of the *X* chromosome expressed in the reproductive tract. Additional experiments show that the preferentially expressed *X* chromosome in the parietal endoderm and visceral yolk sac endoderm of a normal X^mX^p conceptus is always X^m regardless of grandparental origin of X^m and regardless of whether the mother is a normal *XX* female or an *XO* female. X^p is, however, expressed in these tissues in X^pO female conceptuses. It is argued that a form of chromosome imprinting occurs at each generation to mark X^m and X^p as different and that this difference influences the choice of which *X* chromosomes are expressed in each cell lineage.

1. INTRODUCTION

Cytogenetic and genetic studies have shown that *X* chromosome expression is not random in all tissues of the developing female mouse conceptus (Takagi & Sasaki, 1975; West *et al.* 1977; Frels, Rossant & Chapman, 1979; Frels & Chapman, 1980). In some extraembryonic tissues the maternally derived *X* chromosome (X^m) is expressed in preference to its paternal homologue (X^p). This nonrandom *X* chromosome expression seems to be dependent both on the parental origins of the two *X* chromosomes and on the embryonic cell lineage to which the tissue belongs.

By 4½ days *post coitum* (*p.c.*), the mouse embryo comprises three separate tissues,

0016–6723/81/2828–8470 $01.00

the trophectoderm, the primitive endoderm (or hypoblast) and the primitive ectoderm (or epiblast), which form separate cell lineages in later development. Preferential expression of X^m has been reported in two derivatives of the trophectoderm, namely the chorionic ectoderm and the mural trophoblast (Frels, Rossant & Chapman, 1979; Frels & Chapman, 1980) and also in the visceral yolk sac endoderm (West *et al.* 1977), which is the only derivative of the primitive endoderm so far analysed. In contrast, X chromosome expression seems to be more nearly random in the fetus, yolk sac mesoderm and allantois, which are all derived from the primitive ectoderm lineage (Takagi & Sasaki, 1975; West *et al.* 1977; Takagi, 1978). The preferential expression of X^m in the visceral yolk sac is not due to a selective pressure exerted by the maternal environment dependent on X-chromosome-linked gene expression in the mother (West *et al.* 1977). We argued from this that the preferential expression of X^m, in this tissue at least, was due to an intrinsic difference between X^m and X^p, which we described as a difference in 'X chromosome imprinting' between X^m and X^p.

In this study we have continued to examine how nonrandom X chromosome expression is related to cell lineage and to the parental origin of the X chromosomes. Our investigations have been directed towards the following four questions.

(i) Does X chromosome expression in other derivatives of primitive ectoderm and primitive endoderm, namely the amnion and parietal endoderm, fit the lineage dependency model outlined above?

(ii) Is X^m preferentially expressed in the primitive endoderm lineage irrespective of whether it is derived from the maternal grandmother or the maternal grandfather and irrespective of the environment of the reproductive tract? We have analysed the relevant tissues from heterozygous PGK-1AB female conceptuses from reciprocal backcross matings and from embryo transfer and ovary transplant experiments.

(iii) Is the ratio of autosomes to X chromosomes in the diploid germ cells of the parents important in setting up the differential between X^m and X^p? Some kind of interaction between the autosomes and X chromosomes might be one way of introducing a difference in X chromosome imprinting between X^m and X^p since the ratio of autosomes to X chromosomes is different in males and females. If so, there should be no differential between X^m and X^p when X^m is derived from an XO mother, and so preferential expression of X^m would not be expected in these individuals.

(iv) In XO conceptuses, where the only X chromosome is derived from the father, is X^p expressed in tissues that would normally show exclusive or preferential expression of X^m?

2. MATERIALS AND METHODS

(i) *Mice*

The two electrophoretic variants of X-linked phosphoglycerate kinase (PGK-1) described by Nielsen & Chapman (1977) were used as markers for X chromosome expression. Heterozygous *Pgk-1*^{a}/*Pgk-1*^{b} female conceptuses were produced by

reciprocal crosses and reciprocal backcrosses between the random-bred CFLP strain (PGK-1B) and the inbred C3H/HeHa-Pgk-1^a/Ws strain (PGK-1A). The CFLP mice were maintained in Oxford and were derived from mice originally supplied by Anglia Laboratory Animals, Ltd. The C3H/HeHa-Pgk-1^a/Ws strain was derived from a cross between a single *Pgk-1*^{a}/Y male and a C3H/HeHa female. Heterozygous *Pgk-1*^{a}/*Pgk-1*^{b} females were then backcrossed to C3H/HeHa males for a further seven generations to N8 before intercrossing to establish the inbred strain.

A stock of mice homozygous for *Pgk-1*^{b} and the *X* chromosome inversion *In*(*X*) *1H* (Evans & Phillips, 1975) was obtained from R. Phillips at Harwell. *In*(*X*)*1H*/Y males were mated with CFLP females to produce *In*(*X*)*1H*/+ heterozygotes. Some of the gametes produced by the *In*(*X*)*1H*/+ heterozygous females lack an X chromosome. *XO* mice and conceptuses for analysis were then produced in the next three generations from the following matings:

(1) *Pgk-1*^{b}, *In*(*X*)*1H*/*Pgk-1*^{b}, +♀ × *Pgk-1*^{a}, +/Y♂,
(2) *Pgk-1*^{a}/O♀ × *Pgk-1*^{b}/Y♂,
(3) *Pgk-1*^{b}/O♀ × *Pgk-1*^{a}/Y♂.

The males were either CFLP or C3H/HeHa-Pgk-1^a/Ws depending on the *Pgk-1* allele required. The *Pgk-1*^{a}/O and *Pgk-1*^{b}/O mothers used in crosses 2 and 3 above were identified by typing peripheral blood for PGK-1. An additional blood sample and a liver sample were taken when the females were killed for recovery of conceptuses. Six of the mothers that were presumed to be Pgk-1^b/O by electrophoresis of blood and liver samples were confirmed as *XO* by chromosome counts kindly done by E. P. Evans, M. Burtenshaw and B. Brown. (There was one additional case where an adult female was classified as PGK-1B from the blood sample, PGK-1AB from the liver and *XX* by chromosomes. Presumably, she was a PGK-1AB *XX* female but the PGK-1A cell population was absent or at a low level in the blood when the sample was taken.)

In addition, C3H/HeH mice (PGK-1B) from the Zoology Department, Oxford were used in experiments involving ovary transplants. CFLP females were used as recipient foster mothers for embryo transfers.

(ii) *Embryo transfers and ovary transplants*

Blastocysts were flushed from the uterus at $3\frac{1}{2}$ days *p.c.* in PB1 medium plus 10% fetal calf serum (PB1 + 10% FCS). (This medium is essentially the PB1 medium described by Whittingham & Wales (1969) but the original recipe, based on Dulbecco's phosphate buffered saline, was modified by substituting glucose (1 g/l) for lactate and 10% fetal calf serum for bovine serum albumin.) The embryos were then transferred surgically to the uteri of CFLP females on the third day of pseudopregnancy as described by McLaren & Michie (1956), using Avertin anaesthesia.

Ovaries were taken from freshly killed females and were transplanted (either unilaterally or bilaterally) into recipient congenic females under Avertin anaesthesia.

Whole or half ovaries were placed inside the ovarian bursa immediately after removal of the host ovary. In some unilateral transplants, the contralateral ovary was also removed.

(iii) *Dissections and sample preparations*

Conceptuses were removed from the uterus at 12½ days or 17½ days *p.c.* and various tissues were dissected out in PB1 + 10 % FCS under a dissecting microscope. The fetus and visceral yolk sac were dissected at both ages, but the parietal

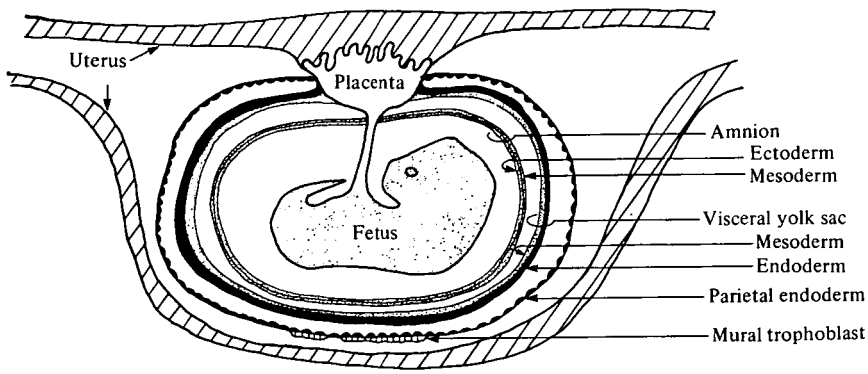

Fig. 1. Diagram showing the arrangement of the fetus and extra-embryonic membranes at about 12½ days. The layers are shaded according to the lineage relationships described by Gardner & Papaioannou (1975). The stippled areas belong to the primitive ectoderm lineage, the black regions are descended from the primitive endoderm and the areas shaded with vertical stripes develop from the trophectoderm. The maternal uterus is shaded with diagonal stripes and the umbilical cord and placenta (of mixed origin) are unshaded.

endoderm was recovered only at 12½ days and the amnion was analysed only at 17½ days *p.c.* The arrangement of these tissues in a mid-gestation conceptus is shown diagrammatically in Fig. 1. The parietal endoderm at 12½ days was covered with a variable amount of mural trophoblast tissue which was carefully picked off with watchmaker's forceps. The endoderm and mesoderm layers of the visceral yolk sac from some conceptuses were separated by dissection following digestion with a mixture of 0·5 % trypsin and 2·5 % pancreatin in calcium- and magnesium-free Tyrode's solution for 3 h at 4 °C (Levak-Švajger, Švajger & Škreb, 1969) followed by up to 2 h at 4 °C in PB1 + 10 % FCS. The larger samples were homogenized in distilled water (30 μl for 12½ day intact visceral yolk sacs and 100–200 μl for 17½ day fetuses). The smaller samples were lysed in 2 μl of distilled water (parietal endoderm, amnion, and visceral yolk sac mesoderm) or 2–10 μl of distilled water (visceral yolk sac endoderm) in a microtest plate. Maternal liver samples were homogenized in distilled water and maternal blood was stored untreated. All samples were coded and stored at − 20 °C before electrophoresis. The larger samples were centrifuged to remove cell debris immediately before electrophoresis.

Only *Pgk-1*^{a}/*Pgk-1*^{b} heterozygotes or *XO* female conceptuses were considered in the experimental analysis. These were identified by their PGK-1 phenotype after

electrophoresis. In some cases the *XX* females were identified either by fetal morphology at $17\frac{1}{2}$ days or by positive sex chromatin staining of the amnion with aceto-orcein (Vickers, 1967) at $12\frac{1}{2}$ days. Males could, therefore, often be discarded before electrophoresis although some were included to avoid prejudicing the blind scoring of the gel patterns.

(iv) *Electrophoretic analysis*

Starch gel electrophoresis was carried out with 12% electrostarch using a pH 6·4 Tris-citrate buffer system. The staining mixture was based on that described by Beutler (1969) and is similar to the one we previously used (West *et al.* 1977) except that a mixture of glycerol-3-phosphate dehydrogenase and triose phosphate isomerase (GDH/TIM from Boehringer) was added (Harris & Hopkinson, 1976) and the NADH concentration was reduced (Cooper *et al.* 1971; Chapman & Frels, personal communication) in order to increase the sensitivity. (20 μl of GDH/TIM mixture (2 mg/ml) and 2 mg NADH were used per 20 ml stain made up in buffer and agar as previously described.) Bands of PGK-1 activity were observed under long-wavelength ultraviolet illumination and the proportions of the two allozyme bands of PGK-1 were classified (blind) by two observers according to the semi-quantitative five-point scale described in Table 1.

3. RESULTS

(i) *X chromosome expression in the amnion and parietal endoderm*

The mean PGK scores from tissues of PGK-1AB conceptuses produced by reciprocal crosses (Table 1) and by crosses involving *In(X)1H/+* heterozygous mothers (crosses 1*a* and 2*a* in Table 3) show that the maternally derived *Pgk-1* allele is preferentially expressed in the parietal endoderm whereas the two *Pgk-1* alleles are expressed more equally in the amnion. In these crosses the mean PGK score is close to 3 for both the fetus and the amnion. The similarity between the PGK scores for the amnion and fetus is shown on a per conceptus basis in Fig. 2, and this is considered in more detail in the Discussion section below. The scores for the parietal endoderm are close to 5 when the *Pgk-1*^{a} allele is inherited from the mother and close to 1 when *Pgk-1*^{b} is inherited from the mother. The PGK scores for whole yolk sac and especially yolk sac endoderm also confirm previous observations on the preferential expression of the maternally derived allele in this tissue (West *et al.* 1977).

(ii) *Effect of grandparental origin of X chromosome and reproductive tract environment on X chromosome expression in the parietal endoderm and visceral yolk sac*

The four reciprocal backcross matings shown in Table 1 confirm that the preferential expression of *Pgk-1* in the visceral yolk sac and parietal endoderm is dependent on the parental origin and not on the uterine environment or on some grandparental effect. To ensure that *Pgk-1* expression in the part of the uterus closest to the conceptus was indeed heterozygous we analysed decidual tissue from

nine implantation sites from a pregnant *Pgk-1*^{a}/*Pgk-1*^{b} heterozygous female on the 9th day of pregnancy. All samples of decidual tissue (which is produced entirely by the uterus) contained both PGK-1A and PGK-1B allozymes, confirming that the uterine environment was mosaic. The maternally derived *Pgk-1* allele is preferentially expressed in the whole visceral yolk sac and parietal endoderm in conceptuses from all four backcrosses shown in Table 1. Since X^m is inherited from

Table 1. *Mean PGK scores in tissues derived from the primitive ectoderm and primitive endoderm in PGK-1AB conceptuses from reciprocal crosses and backcrosses*

(The PGK score is based on a five-point scale: (1) only PGK-1B detected; (2) PGK-1B > PGK-1A; (3) PGK-1B ≃ PGK-1A; (4) PGK-1B < PGK-1A; (5) only PGK-1A detected.)

Cross*	Conceptuses			Primitive ectoderm lineage		Primitive endoderm lineage	
♀ × ♂	X^m†	Age (days)	No.	Fetus	Amnion	Whole yolk sac	Parietal endoderm
				Reciprocal crosses			
BB × AY	B	12½	30	3·30 ± 0·08‡	—	1·97 ± 0·09	1·10 ± 0·05§
BB × AY	B	17½	29	3·17 ± 0·07	3·14 ± 0·12	1·97 ± 0·06	—
AA × BY	A	12½	16	3·25 ± 0·11	—	4·63 ± 0·12	4·94 ± 0·06‖
AA × BY	A	17½	33	3·30 ± 0·09	3·27 ± 0·09	4·39 ± 0·09	—
				Reciprocal backcrosses			
BA × AY	B	12½	10	3·80 ± 0·13	—	1·90 ± 0·22	1·10 ± 0·09¶
AB × AY	B	12½	7	2·71 ± 0·17	—	1·71 ± 0·17	1·14 ± 0·13**
BA × BY	A	12½	11	3·45 ± 0·15	—	4·73 ± 0·13	5·00 ± 0
AB × BY	A	12½	11	3·00 ± 0	—	4·36 ± 0·14	5·00 ± 0

* BB = *Pgk-1*^{b}/*Pgk-1*^{b}♀; BY = *Pgk-1*^{b}/Y♂ (CFLP strain).
AA = *Pgk-1*^{a}/*Pgk-1*^{a}♀; AY = *Pgk-1*^{a}/Y♂ (C3H/HeHa-Pgk-1^a/Ws strain).
BA = (CFLP♀ × C3H/HeHa-Pgk-1^a/Ws ♂) F1♀.
AB = (C3H/HeHa-Pgk-1^a/Ws♀ × CFLP ♂) F1♀.
† B = *Pgk-1*^{b} allele inherited on X^m; A = *Pgk-1*^{a} inherited on X^m.
‡ Mean PGK score ± standard error of the mean.
§ 27 scores of 1 and 3 scores of 2.
‖ 15 scores of 5 and 1 score of 4.
¶ 9 scores of 1 and 1 score of 2.
** 6 scores of 1 and 1 score of 2.

the maternal grandmother in the first and fourth backcross and from the maternal grandfather in the other two backcrosses we can rule out the possibility that the grandparental origin of X^m influences the randomness of its expression. The mean PGK scores for the fetuses are again consistent with random X chromosome expression. Although the variation in mean PGK scores for the reciprocal backcross fetuses is greater than for the fetuses from the reciprocal crosses in Table 1, there is no correlation between preferential X chromosome expression in the fetuses and

either parental or grandparental origin of that X chromosome. (The significantly higher PGK scores for fetuses from PGK-1BA compared with PGK-1AB mothers are not understood but could be an artifact of small sample size and the use of random-bred mice.)

The embryo transfers and ovary transplant experiments shown in Table 2 provide further evidence against a role for selection dependent on the X-chromosome-linked gene expression in the maternal environment. In both of these

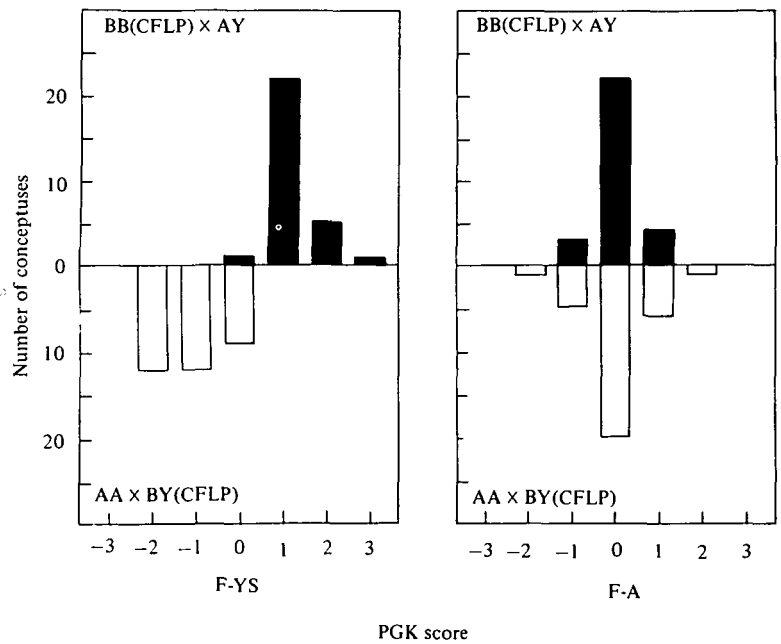

Fig. 2. Distribution of PGK scores in the reciprocal crosses analysed at 17½ days shown in Table 1. The PGK score for each yolk sac (YS) is subtracted from the PGK score for its fetus (F) and plotted as the F-YS difference. Similarly the PGK score for each amnion (A) is subtracted from the fetal PGK score and plotted as the F–A difference The distributions for the AA × BY cross have been inverted so that distributions from reciprocal crosses could be plotted on the same horizontal axis for easier comparison of their positions along that axis. The yolk sac consistently contains a higher proportion of the maternally derived PGK allozyme than the fetus, so the F–YS distributions are skewed away from zero. However, the F–A distributions are approximately normal, with modes and means close to zero, because the proportions of the two allozymes in the amnion are similar to that in the fetus.

experiments the uterine selection pressure postulated to cause preferential expression of X^m would be of opposite influence to that of the genetic mother. Blastocysts, produced by a C3H/HeHa-Pgk-1^a/Ws mother and a CFLP strain father were transferred to a pseudopregnant CFLP strain female. At 12½ days the *Pgk-1*^{a} allele was preferentially expressed in the parietal endoderm and the visceral yolk sac endoderm of PGK-1AB conceptuses even though both X^p and the

https://doi.org/10.1017/S0016672300020152 Published online by Cambridge University Press

Table 2. *Mean PGK scores in PGK-1AB conceptuses derived from embryo transfers and ovary transplants. The five-point scale used for the PGK score is as described for Table 1*

Cross* ♀ × ♂	X^m of conceptuses†	Recipient foster mother*	Primitive ectoderm lineage: Fetus	Primitive ectoderm lineage: Yolk sac mesoderm	Primitive endoderm lineage: Yolk sac endoderm	Primitive endoderm lineage: Parietal endoderm
			Embryo transfers			
AA × BY (CFLP)	A	BB (CFLP)	3·13 ± 0·12 (8)‡	3·25 ± 0·15 (8)	4·88 ± 0·12 (8)§	5·00 ± 0 (8)
			Ovary transplants			
AA × BY(C3H/HeH)	A	BB (C3H/HeH)	3·67 ± 0·27 (3)	3·00 ± 0 (3)	5·00 ± 0 (3)	5·00 ± 0 (3)
BB (C3H/HeH) × AY	B	AA	3·00 ± 0 (2)	4·00 ± 0 (2)	1·00 ± 0 (2)	1·00 (1)

* AA = *Pgk-1*^{a}/*Pgk-1*^{a} ♀; AY = *Pgk-1*^{a}/Y ♂ (C3H/HeHa-Pgk-1^a/Ws strain)
BB = *Pgk-1*^{b}/*Pgk-1*^{b} ♀; BY = *Pgk-1*^{b}/Y ♂ (strains shown in table)
The recipient foster mother receives either blastocysts or ovaries from a different strain.

† B = *Pgk-1*^{b} allele inherited on X^m. A = *Pgk-1*^{a} allele inherited on X^m.

‡ Mean PGK score ± standard error of the mean (number scored).

§ 7 scores of 5 and 1 score of 4.

Table 3. *Mean PGK scores in tissues from PGK-1AB conceptuses and XO litter mates. The five point scale used for the PGK score is as described for Table 1*

Cross*	Conceptuses			Primitive ectoderm lineage			Primitive endoderm lineage		
♀ × ♂	X^m †	Age (days)	No.	Fetus	Amnion	Yolk sac mesoderm	Whole yolk sac	Yolk sac endoderm	Parietal endoderm
				PGK-1AB conceptuses (XX)					
1*a* BB (INX/+) × AY	B	12½	17	2·94 ± 0·15‡	—	—	2·18 ± 0·15	—	1·00 ± 0 (15/17)
2*a* BB (INX/+) × AY	B	17½	25	3·36 ± 0·12	3·48 ± 0·10	3·00 ± 0·10	—	1·08 ± 0·05§	—
3*a* AO × BY	A	12½	10	3·50 ± 0·16	—	3·80 ± 0·13	—	4·86 ± 0·13‖ (7/10)	5·00 ± 0 (9/10)
4*a* BO × AY	B	12½	15	3·00 ± 0	—	3·08 ± 0·28 (13/15)	—	1·00 ± 0	1·00 ± 0 (9/15)
				Presumed X^pO conceptuses					
1*b* BB (INX/+) × AY	O	12½	5	5·00 ± 0	—	—	5·00 ± 0	—	5·00 ± 0
2*b* BB (INX/+) × AY	O	17½	7	5·00 ± 0	5·00 ± 0	5·00 ± 0	—	5·00 ± 0	—
3*b* AO × BY	O	12½	8	1·00 ± 0	—	1·00 ± 0	—	1·00 ± 0(5/8)	1·14 ± 0·13(7/8)¶
4*b* BO × AY	O	12½	11	5·00 ± 0	—	5·00 ± 0	—	5·00 ± 0(10/11)	5·00 ± 0(9/11)

* INX/+ = *In(X)1H*/+ heterozygous ♀; AO = *Pgk1*^{a}/O (*XO*) ♀; BO = *Pgk-1*^{b}/O (*XO*) ♀; BB = *Pgk-1*^{b}/*Pgk-1*^{b}♀; BY = *Pgk-1*^{b}/Y (CFLP strain) ♂; AY = C3H/HeHa-Pgk-*1*^{a}/Ws ♂.

† B = *Pgk-1*^{b} allele inherited on X^m; A = *Pgk-1*^{a} inherited on X^m; O = no maternally inherited *X* chromosome.

‡ Mean PGK score ± standard error of the mean (proportion of samples that were scored). Where the proportion of samples scored is not shown all of the samples were scored. Six of the seven unscoreable parietal endoderm samples in cross 4*a* were run on the same gel and the entire gel (14 samples) stained weakly. Similarly the six unscoreable visceral yolk sac endoderm samples in crosses 3*a* and 3*b* were all run together. In this case all six samples showed PGK-1 activity but the allozymes were not adequately resolved.

§ 23 scores of 1 and 2 scores of 2.

‖ 6 scores of 5 and 1 score of 4.

¶ 6 scores of 1 and 1 score of 2.

recipient female were of the CFLP strain. In the reciprocal ovary transplants between inbred strains, when the whole of development from fertilization occurred in a reproductive tract genetically similar to X^p, the maternally derived *Pgk-1* allele is still preferentially expressed in the parietal endoderm and visceral yolk sac endoderm.

(iii) *X chromosome expression in XX conceptuses with XO mothers*

Analysis of PGK-1 expression in heterozygous XX conceptuses with XO mothers is shown in Table 3. The PGK scores for parietal endoderm and visceral yolk sac show that preferential expression of the maternally derived *Pgk-1* allele occurs just as clearly in these tissues in XX conceptuses with XO mothers (crosses $3a$ and $4a$ in Table 3) as in those with XX mothers (crosses $1a$ and $2a$ in Table 3 and Tables 1 & 2).

(iv) *X chromosome expression in X^pO conceptuses*

The results from X^pO conceptuses produced in crosses $1b$–$4b$ (Table 3) show that PGK-1 activity is detected in all tissues analysed including the parietal endoderm and visceral yolk sac endoderm. Throughout the entire study some samples have been unscoreable either because the PGK-1 activity was too low (usually only amnion or parietal endoderm samples) or because of poor resolution of PGK-1A and PGK-1B bands, but taken together the proportion of scoreable visceral yolk sac endoderm and parietal endoderm samples from X^pO conceptuses (43/50) was not significantly different than from XX conceptuses (80/92): $\chi^2 = 0{\cdot}03$, $P > 0{\cdot}05$. Conceptuses with some unscoreable samples have been omitted from Tables 1 and 2 but included in Table 3, where the proportion of scoreable samples is shown in parentheses after the mean and standard error.

4. DISCUSSION

(i) *Lineage dependency of preferential expression of X^m*

The results of this study support the lineage dependency model for preferential expression of X^m. The fetus shows random expression of X^m and X^p. Of the other tissues that develop from the primitive ectoderm lineage, the allantois (Takagi & Sasaki, 1975) and the visceral yolk sac mesoderm (West *et al.* 1977) both showed a slight preferential expression of X^m in the first experiments reported. Later studies involving larger numbers of allantois samples (Takagi, 1978) and individual rather than pooled visceral yolk sac mesoderm fractions (Table 3) support a random expression of X^m and X^p in these tissues. Our present results for the amnion now add a fourth tissue from this lineage that shows essentially random expression of X^m and X^p.

We have presented the results in the tables in terms of a mean PGK score for each group. However, in those tissues where both X^m and X^p are usually expressed, it is helpful to consider the individual PGK scores for these tissues relative to the

corresponding PGK score for the fetus. Comparison of PGK scores from two tissues from the same conceptus parcels out any genetic variation among individuals that influences the randomness of X chromosome expression. The data for the $17\frac{1}{2}$ day conceptuses from reciprocal crosses (Table 1) is plotted on a per conceptus basis in Fig. 2. The PGK score for the whole visceral yolk sac has been subtracted from the score for the corresponding fetus and plotted as the fetus minus yolk sac (F-YS) PGK score. Similarly the fetus minus amnion (F-A) PGK score is plotted to show any difference between the fetus and its amnion. The preferential expression of X^m in the whole visceral yolk sac is reflected by the positions of the distributions for F-YS which are skewed in opposite directions for reciprocal crosses. However, both of the F-A distributions approximate normal distributions with modes (and means) of 0, indicating that in most conceptuses the X chromosome expression is similar in the fetus and the amnion.

The amnion comprises two layers (the ectoderm and the mesoderm) both of which are derived from the primitive ectoderm lineage of the mouse blastocyst (Gardner & Papaioannou, 1975). The visceral yolk sac is also composed of two layers, but the endoderm layer is derived from the primitive endoderm and the mesoderm layer belongs to the primitive ectoderm lineage (Gardner & Papaioannou, 1975). Previous work (West *et al.* 1977) and results from this study (Table 2 and crosses $2a$–$4a$ in Table 3) show that preferential expression of the maternally derived allele in the visceral yolk sac is the consequence of almost exclusive expression in the visceral yolk sac endoderm and a more equal expression in the visceral yolk sac mesoderm. The amnion ectoderm and mesoderm were not separated but the results of the amnion are clearly different from the visceral yolk sac and suggest that consistent preferential expression of one allele does not occur in either layer of the amnion unless a preferential expression of the maternally derived allele in one layer is either balanced by a preferential expression of the other allele in the second layer, or masked by a much higher PGK-1 activity in the second layer. It seems more likely that X chromosome expression is random in both layers of the amnion.

The parietal endoderm and the visceral yolk sac endoderm showed only the maternally derived PGK-1 allozyme in the great majority of cases in the present study, indicating an overwhelming preference for the expression of X^m. It is still not clear whether the presence of the paternally derived PGK-1 allozyme in some samples is the result of a false positive due to technical reasons or whether this indicates that X^m is preferentially expressed but not always exclusively expressed in these tissues. However, it is clear that neither of these derivatives of the primitive endoderm lineage shows random expression of X^m and X^p.

These results on parietal endoderm and visceral yolk sac endoderm, together with the work from other laboratories on other tissues (Takagi, 1978; Frels & Chapman, 1980; Frels, Rossant & Chapman, 1979) supports the idea that X chromosome expression is random in the primitive ectoderm lineage but that X^m is preferentially (and possibly exclusively) expressed in the primitive endoderm and trophectoderm lineages.

(ii) *Influence of reproductive tract and grandparental origin*

In both the parietal endoderm and the visceral yolk sac endoderm, X^m is preferentially expressed regardless of the oviduct or uterine environment. We previously argued from similar experiments on whole visceral yolk sacs that this implies that X^m and X^p are somehow 'marked' as different from one another and that this differential 'marking' (or chromosome imprinting) influences which X chromosome is expressed (West *et al.* 1977). This imprinting could occur at any time before or during fertilization. The results from reciprocal backcrosses show that X^m is preferentially expressed in these tissues regardless of whether X^m is inherited from the maternal grandmother or the maternal grandfather, which implies that the differential imprinting occurs anew at each generation.

In addition, these experiments, where X^m is preferentially expressed in the parietal endoderm and visceral yolk sac endoderm despite the expression of a different *Pgk-1* allele in the reproductive tract, show that this preferential expression is not an artifact resulting from maternal contamination. However, without sophisticated blastocyst reconstitution experiments (Gardner, Papaioannou & Barton, 1973) it is impossible to rule out completely that the PGK-1 activity from the parietal endoderm samples is due to contamination from the overlying mural trophoblast.

(iii) *Importance of the X chromosome constitution of the mother*

An interaction between the autosomes and the X chromosomes in the diploid germ cells would be one way of imprinting X^m and X^p differently since the ratio of autosomes to X chromosomes differs between normal males and females. If so the imprinting of the X chromosome in an XO germ cell would be similar to the normal X^p rather than the normal X^m. However, since the X chromosome inherited from XO mothers is preferentially expressed in XX progeny despite the similar autosome:X chromosome ratio in the maternal and paternal diploid germ cells this can be excluded as a mechanism for achieving a differential imprinting of X^m and X^p.

(iv) X^pO *conceptuses*

The difference between X^m and X^p probably causes the preferential expression of X^m by controlling the randomness of X chromosome inactivation in certain tissues. This could result if the differential chromosome imprinting of X^m and X^p acted either (*a*) by programming X^p to inactivate in certain tissues or (*b*) by influencing the choice of which X chromosome is inactivated without providing the primary signal for X chromosome inactivation to occur. If the first of these two possibilities is true we would expect X^p to be inactivated in the visceral yolk sac endoderm and parietal endoderm of X^pO conceptuses, and we would not expect to detect any new X-linked gene product after X chromosome inactivation had occurred. Our results, however, show that X^p is consistently expressed in these tissues in X^pO females. Enzyme activity studies were not done, but neither the staining intensity nor the proportion of scoreable samples of the tissues was significantly lower in X^pO conceptuses than in XX or XY individuals at the same

developmental stage. The expression of X^p in the mural trophoblast of X^pO individuals has also been reported recently (Frels & Chapman, 1979). Unless there is a selection pressure to reactivate an inactive X^p or to repopulate the entire trophectoderm and primitive endoderm lineages from a few cells, this suggests that X^p is not inactivated in X^pO individuals even in those tissues where X^m is normally almost exclusively expressed. If so, this suggests that the differential imprinting of X^m and X^p influences the choice of which X chromosome is to be inactivated but does not provide the primary signal for X chromosome inactivation to occur.

(v) *X chromosome imprinting and inactivation*

The concept that non-random X chromosome inactivation may result from differential X chromosome imprinting was developed by Cooper (1971) and Brown & Chandra (1973). However, these authors argued that X chromosome imprinting provided the primary signal for X chromosome inactivation to occur, whereas we invoke X chromosome imprinting simply as a modifier of an inactivation event that is initiated independently. This issue is also discussed by Kaufman, Guc-Cubrilo & Lyon (1978) who reach a similar conclusion. The nature of this X chromosome imprinting is unknown. It could affect the entire X chromosome or modify a specific site. One possibility is that the imprinting represents a gross physiological alteration of the *Xce* region, since genetic polymorphisms of this locus can influence the randomness of X chromosome expression in the adult (reviewed by Cattanach, 1975) and probably the fetus (West & Chapman, 1978).

It is still not clear how the differential X chromosome imprinting influences the randomness of X chromosome inactivation in a lineage-specific way. Our current thinking is influenced by Lyon (1977) who suggested that the differential in X chromosome imprinting may 'wear off' during the period of early mouse development and that X chromosome inactivation may occur at different times in different tissues so that the effect of the imprinting is lost by the time X chromosome inactivation occurs in some tissues. This idea has been discussed in relation to the three primary lineages of early mouse development by Takagi (1978), West *et al.* (1978) and Monk & Harper (1979). In order to fit Lyon's (1977) suggestion to our present results we would require that X chromosome inactivation occurs later in the primitive ectoderm lineage than in the other two lineages. This has yet to be proved, but the recent results of Monk & Harper (1979) support this idea, and other more circumstantial evidence has been discussed by West *et al.* (1978).

Analysis of earlier embryos, using either cytogenetic techniques or a more sensitive X-linked genetic marker, may be necessary before the control of the randomness of X chromosome expression can be more fully understood.

We wish to thank Teresa Clements for excellent technical help with the electrophoresis and Ted Evans, Mike Burtenshaw and Beverly Brown for kindly doing chromosome counts on some of our XO mice. We also thank Verne Chapman and Bill Frels for many hours of helpful discussion and in particular for suggesting the experiment involving XX conceptuses with XO mothers. We are also grateful to Verne Chapman, Rita Phillips and Chris Graham for kindly supplying mice, to Richard Gardner and Teresa Clements for their comments on the manuscript and to the MRC for financial support.

REFERENCES

BEUTLER, E. (1969). Electrophoresis of phosphoglycerate kinase. *Biochemical Genetics* **3**, 189–195.

BROWN, S. W. & CHANDRA, H. S. (1973). Inactivation system of the mammalian X chromosome. *Proceedings of the National Academy of Sciences, USA* **70**, 195–199.

CATTANACH, B. M. (1975). Control of chromosome inactivation. *Annual Review of Genetics* **9**, 1–18.

COOPER, D. W. (1971). Directed genetic change model for X chromosome inactivation in eutherian mammals. *Nature* **230**, 292–294.

COOPER, D. W., VANDEBERG, J. L., SHARMAN, G. B. & POOLE, W. E. (1971). Phosphoglycerate kinase polymorphism in kangaroos provides further evidence for paternal X-inactivation. *Nature New Biology* **230**, 155–157.

EVANS, E. P. & PHILLIPS, R. J. S. (1975). Inversion heterozygosity and the origin of XO daughters of *Bpa*/+ female mice. *Nature* **256**, 40–41.

FRELS, W. I. & CHAPMAN, V. M. (1979). Paternal X chromosome expression in extraembryonic membranes of XO mice. *Journal of Experimental Zoology* **210**, 553–560.

FRELS, W. I. & CHAPMAN, V. M. (1980). Expression of the maternally derived X chromosome in the mural trophoblast of the mouse. *Journal of Embryology and Experimental Morphology* **56**, 179–190.

FRELS, W. I., ROSSANT, J. & CHAPMAN, V. M. (1979). Maternal X chromosome expression in mouse chorionic ectoderm. *Developmental Genetics* **1**, 123–132.

GARDNER, R. L. & PAPAIOANNOU, V. E. (1975). Differentiation in the trophectoderm and inner cell mass. In *The Early Development of Mammals*, Second Symposium of the British Society for Developmental Biology (ed. M. Balls and A. E. Wild), pp. 107–132. Cambridge University Press.

GARDNER, R. L., PAPAIOANNOU, V. E. & BARTON, S. C. (1973). Origin of the ectoplacental cone and secondary giant cells in mouse blastocysts reconstituted from isolated trophoblast and inner cell mass. *Journal of Embryology and Experimental Morphology* **30**, 561–572.

HARRIS, H. & HOPKINSON, D. A. (1976). *Handbook of Enzyme Electrophoresis in Human Genetics*. Amsterdam: North-Holland.

KAUFMAN, M. H., GUC-CUBRILO, M. & LYON, M. F. (1978). X chromosome inactivation in diploid parthenogenetic mouse embryos. *Nature* **271**, 547–549.

LEVAK-ŠVAJGER, B., ŠVAJGER, A. & ŠKREB, N. (1969). Separation of germ layers in presomite rat embryos. *Experientia* **25**, 1311–1312.

LYON, M. F. (1977). Chairman's address. In *Reproduction and Evolution*. Proceedings of the 4th Symposium on Comparative Biology of Reproduction, pp. 95–98. Australian Academy of Science.

MCLAREN, A. & MICHIE, D. (1956). Studies on the transfer of fertilized mouse eggs to uterine foster-mothers. 1. Factors affecting the implantation and survival of native and transferred eggs. *Journal of Experimental Biology* **33**, 394–416.

MONK, M. & HARPER, M. I. (1979). Sequential X chromosome inactivation coupled with cellular differentiation in early mouse embryos. *Nature* **281**, 311–313.

NIELSEN, J. T. & CHAPMAN, V. M. (1977). Electrophoretic variation for sex-linked phosphoglycerate kinase (PGK-1) in the mouse. *Genetics* **87**, 319–325.

TAKAGI, N. (1978). Preferential inactivation of the paternally derived X chromosome in mice. In *Genetic Mosaics and Chimeras in Mammals* (ed. L. B. Russell), pp. 341–360. Basic Life Sciences, volume 12. New York: Plenum Press.

TAKAGI, N. & SASAKI, M. (1975). Preferential inactivation of the paternally derived X chromosome in the extraembryonic membranes of the mouse. *Nature* **256**, 640–642.

VICKERS, A. D. (1967). Amniotic sex chromatin and foetal sexing in the mouse. *Journal of Reproduction and Fertility* **14**, 503–505.

WEST, J. D. & CHAPMAN, V. M. (1978). Variation for X chromosome expression in mice detected by electrophoresis of phosphoglycerate kinase. *Genetical Research* **32**, 91–102.

WEST, J. D., FRELS, W. I., CHAPMAN, V. M. & PAPAIOANNOU, V. E. (1977). Preferential expression of the maternally derived X chromosome in the mouse yolk sac. *Cell* **12**, 873–882.

WEST, J. D., PAPAIOANNOU, V. E., FRELS, W. I. & CHAPMAN, V. M. (1978). Preferential expression of the maternally derived X chromosome in extraembryonic tissues of the mouse. In *Genetic Mosaics and Chimeras in Mammals* (ed. L. B. Russell), pp. 361–377. Basic Life Sciences, volume 12. New York: Plenum Press.

WHITTINGHAM, D. G. & WALES, R. G. (1969). Storage of two-cell mouse embryos *in vitro*. *Australian Journal of Biological Sciences* **22**, 1065–1068.