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Random X-chromosome inactivation in female primordial germ cells in the mouse

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SUMMARY

The pattern of expression of the two X chromosomes was investigated in pre-meiotic germ cells from 12½-day-old female embryos heterozygous for the variant electrophoretic forms of the X-linked enzyme phosphoglycerate kinase (PGK-1). If such germ cells carry the preferentially active Searle's translocated X chromosome (Lyon, Searle, Ford & Ohno, 1964), then only the *Pgk-1* allele on this chromosome is expressed. This confirms Johnston's evidence (1979, 1981) that *Pgk-1* expression reflects a single active X chromosome at this time. Extracts of 12½-day germ cells from heterozygous females carrying two normal X chromosomes show both the A and the B forms of PGK; since only one X chromosome in each cell is active, different alleles must be expressed in different cells, suggesting that X-chromosome inactivation is normally random in the germ line. This result makes it unlikely that germ cells are derived from the yolk-sac endoderm where the paternally derived X chromosome is preferentially inactivated. In their pattern of X-chromosome inactivation, germ cells evidently resemble other tissues derived from the epiblast.

INTRODUCTION

During development of the female mouse embryo, X-chromosome inactivation (Lyon, 1961) is non-random in extraembryonic tissues derived from the trophectoderm and the primary endoderm. The paternally inherited X chromosome is preferentially inactivated (Takagi & Sasaki, 1975; West, Frels, Chapman & Papaioannou, 1977). Later, in the embryo proper, X-chromosome inactivation appears to be random. It has been suggested (Lyon, 1977; Monk, 1978) that whether or not inactivation is random in a particular tissue depends on the interval elapsing between fertilization and the origin of the cell lineage for that tissue.

Several authors have now demonstrated that female germ cells of foetal mice undergo X-chromosome inactivation followed by reactivation (Andina, 1978; Johnston, 1979, 1981; Gartler, Rivest & Cole, 1980; Monk & McLaren, 1981; P. Kratzer & V. Chapman, personal communication). Monk & McLaren (1981) have shown by gene-dosage effects on the X-linked hypoxanthine

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phosphoribosyl transferase (HPRT, E.C. 2.4.2.8) that only one X chromosome is expressed in pre-meiotic ($11\frac{1}{2}$ days *post coitum*) germ cells and that reactivation of the silent X occurs just before the cells enter meiotic prophase. Johnston (1979, 1981) has shown that only the allele carried by the preferentially active Searle's X chromosome (*Pgk-1*^{b}) is expressed in $12\frac{1}{2}$-day female germ cells heterozygous for *Pgk-1*^{a} and *Pgk-1*^{b} coding for the A and B forms of X-linked phosphoglycerate kinase (PGK-1, E.C. 2.7.2.3). Hence PGK expression at this stage reflects a single active X chromosome. In $13\frac{1}{2}$-day germ cells the *Pgk-1*^{a} allele is also expressed as X-chromosome reactivation occurs.

It is not known when the germ-cell lineage is set aside during early development, but it is generally believed to be after the delineation of the primary endoderm (see Discussion). If the germ-cell lineage were to arise at or soon after the time of formation of primary endoderm, then it is possible that the paternal X chromosome would be preferentially inactivated irrespective of the tissue in which the lineage arose. In this paper we confirm Johnston's earlier work (above) showing that *Pgk-1* expression in $12\frac{1}{2}$-day female germ cells carrying Searle's translocation reflects the X-inactivated state. Further, we have examined *Pgk-1* expression in germ cells, yolk-sac endoderm, yolk-sac mesoderm and neural ectoderm in $12\frac{1}{2}$-day female embryos carrying two normal X chromosomes and heterozygous for *Pgk-1*. Only the maternally derived isozyme is expressed in the yolk-sac endoderm whereas germ cells, like yolk-sac mesoderm and neural ectoderm, show approximately equal expression of the two PGK isozymes.

MATERIALS AND METHODS

Mice used

The PGK-1A mouse stock (kindly provided by John West) was derived from a feral population of *Mus musculus* (Nielsen & Chapman, 1977) and made congenic with the inbred C3H strain (West & Chapman, 1978). Female mice carrying the X-autosome translocation T(X; 16) 16H (Searle's translocation, Lyon *et al.* 1964) and the *Pgk-1*^{b} allele were kindly provided by Sohaila Rastan. Random-bred MF 1 mice (OLAC) were used as a source of the *Pgk-1*^{b} allele on a normal X chromosome.

Dissections and sample collections

Pregnant females were killed $12\frac{1}{2}$ days following copulation. Embryos were dissected into PB 1 (Whittingham & Wales, 1969) containing 0·4% polyvinylpyrollidone (PVP) instead of albumin. Embryonic gonads were sexed by their characteristic morphology (developing testes show cords).

From females heterozygous for *Pgk-1*, but not carrying Searle's translocation, the following samples were collected. The yolk sac was dissected free of the embryo and the endoderm and mesoderm layers loosened by digestion in a mixture containing 0·5% trypsin (Sigma) and 2·5% pancreatin (BDH) in

calcium- and magnesium-free phosphate-buffered saline (PBS) for 1 h at 4 °C (Levak-Švajger, Švajger & Škreb, 1969). Further enzyme digestion was prevented by placing the yolk sac in PB 1 containing 10 % foetal calf serum (FCS). After ½ h at 4 °C the endodermal and mesodermal layers were separated using watchmakers' forceps. Samples of embryonic neural ectoderm were also collected from the embryos. All tissue samples were washed three times in PBS and stored frozen at −70 °C.

Embryonic germ cells, embryonic liver, maternal liver and maternal bone marrow were collected from matings in which the *Pgk-1*^{b} allele was donated by mice carrying Searle's translocation. All samples were washed three times in PBS and frozen at −70 °C.

Germ cells were collected from all female embryos by the same technique. Gonads were dissected from the embryo, washed three times in PBS and the germ cells isolated by slitting the gonads with electrolytically sharpened tungsten needles and squeezing. Germ cells were then collected in a pulled Pasteur pipette in approximately 1 μl of PBS. Germ cells could be distinguished from the principal somatic cell contamination (blood cells) by their large size and lack of pigmentation.

Evaluation of PGK-1 isozyme expression in tissue samples

All tissue samples, excluding the germ cell collections and the bone marrow samples, were sonicated briefly to lyse the cells, and centrifuged. The germ and marrow cell samples were freeze-thawed three times in liquid nitrogen and centrifuged. Samples of supernatant were analysed by cellogel electrophoresis using the techniques of Bücher *et al.* (1981).

RESULTS

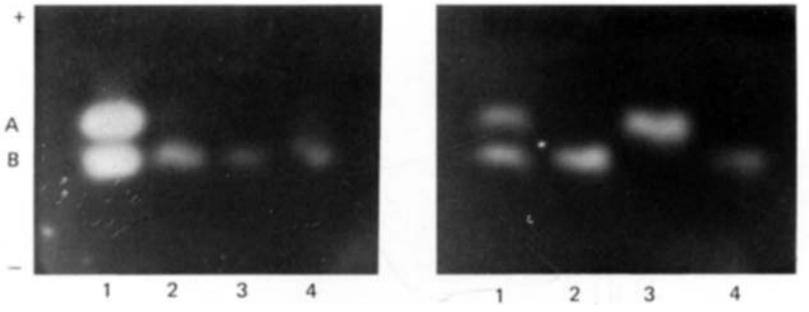
One X chromosome is active in 12½-day female germ cells

We have analysed female embryos heterozygous for *Pgk-1*^{a} and *Pgk-1*^{b} and carrying the Searle's translocated X chromosome to confirm Johnston's (1979, 1981) evidence that a single X chromosome is functional in 12½-day female foetal germ cells. As the translocated X chromosome, which carries the *Pgk-1*^{b} allele, is preferentially active, only the PGK-1B isozyme is expressed in cells that have inactivated an X chromosome. From the mating scheme shown in Table 1, it is apparent that only half the female embryos will carry the translocated X chromosome. The females of this genotype express only the PGK-1B isozyme in somatic cell samples and in germ cells prior to reactivation of the inactive X chromosome carrying the *Pgk-1*^{a} allele. Figure 1*a* shows PGK-1B expression in germ cells and liver from such a female embryo at 12½ days. The sister of this particular embryo was homozygous for *Pgk-1a* which confirms that the mother was indeed heterozygous for *Pgk-1*^{a} and *Pgk-1*^{b} and carried the Searle's translocation (Figure 1*b*).

Table 1. *Mating scheme for obtaining female embryos heterozygous for Pgk-1 and carrying Searle's translocation*

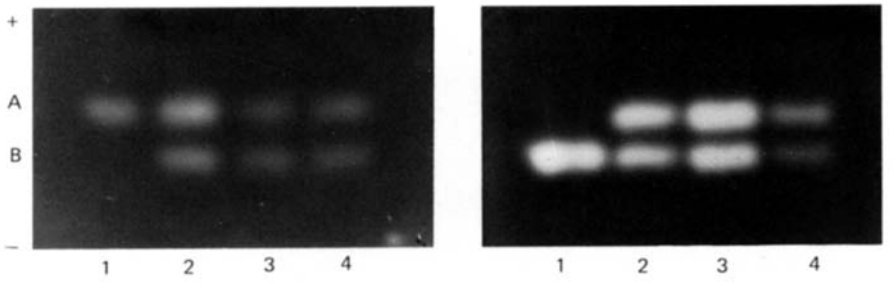
	Mother		Father
	$T16X^b/X^a$	×	X^a/Y
Female progeny	$T16X^b/X^a$	and	X^a/X^a

T16X = Searle's translocation.



(*a*) (*b*)

Fig. 1. PGK expression in 12½-day female embryos from the mating shown in Table 1. (*a*) 1, control PGK-1A and PGK-1B mixture; 2, PGK expression in the $T16X^b/X^a$ mother's liver; 3, PGK expression in a liver sample of 12½-day $T16X^b/X^a$ embryo; 4, PGK expression in the germ cells from the same embryo. (*b*) 1 and 2, as in Fig. 1*a*; 3, PGK expression in the liver of an X^a/X^a sister to the $T16X^b/X^a$ embryo; 4, as 3 in Fig. 1*a*.



(*a*) (*b*)

Fig. 2. PGK expression in tissues from 12½-day heterozygous female embryos. (*a*) Embryo from a *Pgk-1*^{a}♀ × *Pgk-1*^{b}♂ mating. 1, PGK expression in yolk-sac endoderm; 2, PGK expression in yolk-sac mesoderm; 3, PGK expression in neural ectoderm; 4, PGK expression in germ cells. (*b*) Embryo from a *Pgk-1*^{b}♀ × *Pgk-1*^{a}♂ mating. Tissue samples as in Fig. 2*a*.

Table 2. *PGK expression in tissues fom heterozygous 12½-day-old female embryos*

Cross	Embryo	Percentage of maternally derived isozyme			
		Yolk-sac endoderm	Yolk-sac mesoderm	Neural ectoderm	Germ cells
A♀ × B♂	1*	100	64·4 ± 0·6	56·6 ± 0·3	57·8 ± 1·4
A♀ × B♂	2	100	68·2 ± 0·5	54·7 ± 0·7	66·9 ± 0·5
A♀ × B♂	3	100	73·9 ± 0·8	78·3 ± 1·1	66·9 ± 0·7
B♀ × A♂	1*	100	42·7 ± 0·8	46·0 ± 0·4	36·7 ± 0·5
B♀ × A♂	2	100	44·7 ± 0·4	40·8 ± 0·5	40·6 ± 1·3
B♀ × A♂	3	100	50·0 ± 0·8	49·4 ± 1·3	53·6 ± 1·2

* Gels and quantitative recordings for PGK expression in these embryos are shown in Figs. 2*a*, *b* and 3.

PGK expression in 12½-day heterozygous female embryos not carrying the Searle's X

Seven heterozygous embryos from matings between PGK-1B females and PGK-1A males, and six from the reciprocal mating have been examined. Figures 2*a*, *b* show the PGK-1 expression in different tissues of representative embryos from these crosses. In all embryos the yolk-sac endoderm expressed only the maternally inherited PGK-1 isozyme. In contrast the germ cells have a pattern of isozyme expression similar to that of the yolk-sac mesoderm and neural ectoderm, with both A and B forms present.

The quantitation of the two isozymic forms in each tissue was made using Bücher's direct fluorescence technique (Bücher *et al.* 1981; see Fig. 3). The results for six of the embryos thus analysed are shown in Table 2. The consistently higher contribution of the PGK-1A isozyme in the mesoderm, ectoderm and germ-cell samples, whether maternally or paternally derived, is attributed to a high expression Xce allele on the *Pgk-1*^{a} carrying X chromosome (Johnston & Cattanach, 1981). Nevertheless, it is clear that the pattern of X-chromosome inactivation in the germ cells resembles that in epiblast-derived tissues (yolk-sac mesoderm and neural ectoderm) and differs from the yolk-sac endoderm which consistently only expresses the maternally derived allele.

DISCUSSION

Early work based on identification of alkaline-phosphatase-positive germ cells in 8-day-old embryos (Chiquoine, 1954; Mintz & Russell, 1957) showed most of the germ cells associated with yolk-sac endoderm (a derivative of primary endoderm) and it was suggested that the germ-cell lineage derived from this tissue. However Ożdżeński (1967) observed germ cells at an earlier stage at the base of the allantoic rudiment, a mesodermal derivative, and suggested that

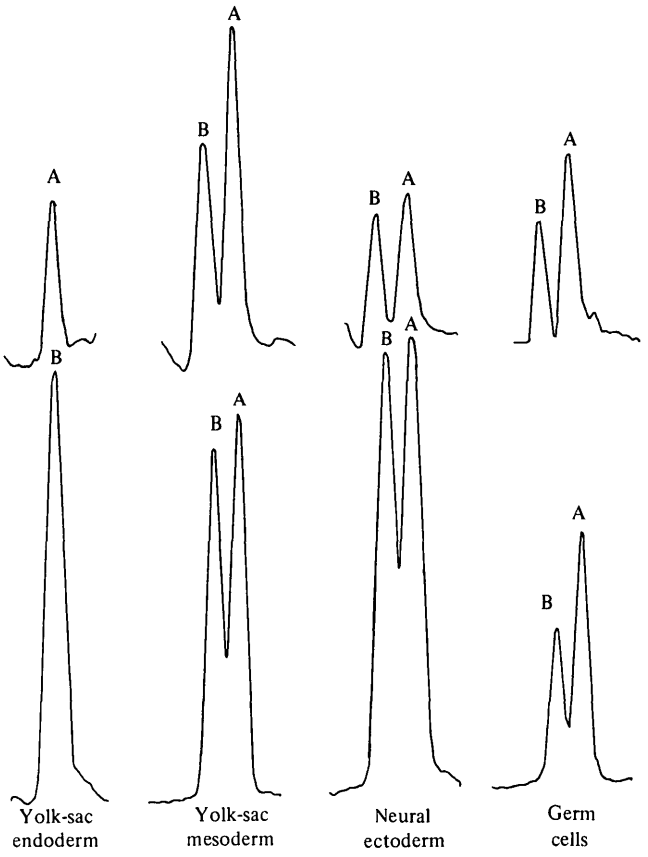


Fig. 3. Quantitation of the PGK-1A and PGK-1B expression for the tissue samples in Fig. 2.

they arose from the primitive streak. In addition, Spiegelman & Bennett (1973) and Clark & Eddy (1975) pointed out that the germ cells bore little morphological resemblance to the cells of the yolk-sac endoderm.

It is now generally believed that the germ cells originate in the epiblast after primary endoderm has formed. Gardner & Rossant (1976) obtained germ-line chimaeras by injection of $4\frac{1}{2}$-day epiblast cells into host blastocysts. At this stage of development the epiblast and primary endoderm are distinct committed cell lineages, the epiblast giving rise to the entire foetus (Gardner & Rossant, 1979). These authors suggest that the germ cells arise in the epiblast and subsequently migrate into extraembryonic endoderm. Further evidence that the germ cells arise in the epiblast comes from the data of Ford *et al.* (1975) who showed a positive correlation in the proportions of two cell types in germ cells and coat pigmentation in chimaeras. Theoretical considerations of Falconer & Avery (1978) predict such a correlation only if the germ cells originate in the epiblast but not in the primary endoderm.

In the work reported here we have compared the pattern of X-chromosome inactivation in germ and somatic cell samples as a marker of cell lineage. The aim was to determine whether the germ-cell lineage resembled the trophectoderm and primary endoderm lineages in expressing only the maternally derived X chromosome, or whether the germ line showed random X-chromosome inactivation similar to the foetus as a whole.

Having confirmed Johnston's earlier finding that only one X chromosome is expressed in pre-meiotic female germ cells at $12\frac{1}{2}$ days *post coitum*, we compared various tissues from heterozygous female embryos from reciprocal crosses for the pattern of expression of *Pgk-1*^{a} and *Pgk-1*^{b}. It was found, in accordance with earlier work (West *et al.* 1977), that the yolk-sac endoderm expresses only the maternally derived isozyme. However, the germ cells at $12\frac{1}{2}$ days *post coitum*, yolk-sac mesoderm and embryonic neural ectoderm express both isozymes. This suggests that germ cells, like other epiblast-derived tissues, show random X-chromosome inactivation, and thus provides further evidence that the germ-cell lineage arises in the epiblast and not in the primary endoderm. The possibility that the germ cells and the three definitive germ layers (ectoderm, mesoderm and endoderm) arise from a common precursor cell pool is currently being investigated.

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