

X-chromosome activity in female mouse embryos heterozygous for *Pgk-1* and Searle's translocation, T(X; 16) 16H

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(Received 19 August 1982)

SUMMARY

To investigate whether the preferential expression of genes on the translocated X chromosome in female mice carrying the X-autosome translocation T(X; 16)16H (Searle's translocation) is due to non-random inactivation or to cell selection, we examined tissues of mouse embryos heterozygous for the X-linked gene coding for phosphoglycerate kinase (*Pgk-1*). From the cross T16HPgk-1^b/+Pgk-1^b ♀ × +Pgk-1^a/Y♂, embryos expressing both isozymic forms of PGK-1 in the epiblast, and only the maternally inherited *Pgk-1^b* allele in extra-embryonic tissues, were assumed to be chromosomally balanced, heterozygous female embryos carrying the Searle's translocation (like the mother). The normal X chromosome in this cross carries a high-expression *Xce^c* locus. At 6 days post-coitum (p.c.) both isozymes were equally expressed in the epiblast as expected if both X chromosomes are active, but by 7 days p.c. the PGK-1B contribution was significantly less than 50%, suggesting that X inactivation has occurred with a bias towards inactivation of the translocated X chromosome carrying the lower-expression *Xce* allele. By 8 days p.c. the situation was the reverse, with a *Pgk-1^b* contribution of significantly more than 50%, and by 12½ days p.c. no *Pgk-1^a* expression could be detected. We interpret the dramatic change in isozyme expression between 7 and 8 days p.c. as indicating rapid selection against cells that had inactivated the translocated 16^X chromosome. Two 7-day p.c. embryos unexpectedly showed equal expression of both *Pgk-1* alleles in both embryonic and extra-embryonic tissues; these were presumably chromosomally unbalanced embryos which had inherited from the mother both an active translocated 16^X chromosome carrying *Pgk-1^b* and an active normal X chromosome carrying *Pgk-1^a*.

1. INTRODUCTION

X-inactivation in cells of female eutherian mammals is generally random (Lyon, 1961) and complete for genes on the inactivated X-chromosome (Lyon, 1966*b*).

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However, non-random *X*-inactivation has been observed in female mammals carrying reciprocal translocations between an *X*-chromosome and an autosome. Several such translocations have been reported in human females (Therman & Patau, 1974; Laurent, Biemont & Dutrillaux, 1975; Leisti, Kabaek & Rimoin, 1975; Hagemeijer *et al.* 1977) in which the inactive *X*-chromosome was identified

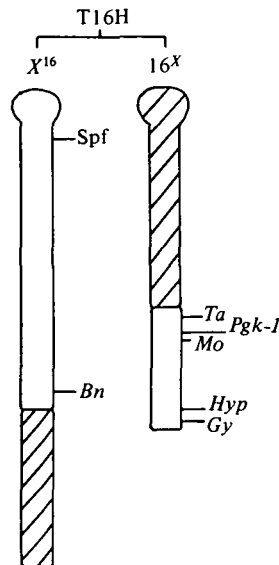


Fig. 1. Diagrammatic representation of Searle's translocation. The shaded area represents chromosome 16 and the unshaded area *X*-chromosome elements. X^{16} is the *X*-chromosome region proximal to the breakpoint carrying the *X*-chromosome centromere and distal region of chromosome 16. 16^X is the chromosome-16 region proximal to the breakpoint carrying the chromosome-16 centromere and distal region of the *X*-chromosome. See Lyon (1966*a*) for mapping of the translocation, Eicher *et al.* (1972) for chromosome assignment and Nielsen & Chapman (1977) for the mapping of *Pgk-1*.

as late replicating (Cohen & Rattazzi, 1971). In most cases the translocated *X*-chromosome was found to be preferentially active. A similar result has been reported for an *X*-autosome translocation in the cow (Gustavsson *et al.* 1968). In the mouse, an *X*-autosome translocation, Searle's translocation (Searle, 1962; Lyon *et al.* 1964), which suppresses sex-linked variegation, has been described. The translocation is a reciprocal one between the *X*-chromosome and chromosome 16 (Eicher, Nesbitt & Francke, 1972), designated T(*X*;16)16H (shortened here to T16H). The breakpoint on the *X* chromosome is slightly proximal to the middle (Eicher *et al.* 1972), between bent tail (*Bn*) and tabby (*Ta*; Lyon, 1966*a*) as shown in Fig. 1. Most, if not all, cells in adult female mice heterozygous for the translocation inactivate the normal *X*-chromosome (Lyon *et al.* 1964; Ohno & Lyon, 1965; Russell & Cacheiro, 1978; Disteche, Eicher & Latt, 1981).

Non-random *X*-chromosome expression in females with *X*-autosome translocations may be produced by two mechanisms:

- (a) Non-random *X*-chromosome inactivation.

(b) Random *X*-chromosome inactivation followed by cell selection against cells inactivating the translocated *X*-chromosome.

Ohno (1967) has suggested that *X*-inactivation of the two separated halves of the *X*-chromosome in Searle's female mice is beyond the means of the dosage compensation mechanism, and therefore *X*-inactivation must be a non-random event. This is not borne out however by other reciprocal *X*-autosome translocations with similar breakpoints which show random *X*-inactivation (Russell & Montgomery, 1970).

Disteche *et al.* (1981) examined late replication by fluorescence staining of chromosomes in 9½-day p.c. Searle's-carrying female embryos. A late replicating normal *X*-chromosome was shown by 99% of cells suggesting that if cell selection does occur it has to take place before 9½ days p.c. Takagi (1980) in a similar study of 6½-, 7½- and 8½-day p.c. embryos reported that most cells of the 6½-day p.c. embryo inactivated the normal *X*-chromosome. As inactivation is thought to occur close to 6 days (Monk & Harper, 1979), this would suggest that *X*-inactivation in the Searle's heterozygotes is primarily non-random.

On the other hand the low birth weight of females heterozygous for Searle's translocation (Lyon *et al.* 1964) may be explained by earlier cell death consistent with cell selection. Extreme patterns of *X*-inactivation observed would result from inactivation of autosomal loci on the translocated regions of chromosome 16, which in the hemizygous state are incompatible with cell survival. In this case, cell selection would occur early in development.

To investigate the origin of the preferential expression, we have analysed *X*-chromosome activity in 6-, 7-, 8- and 12-day p.c. embryos heterozygous for Searle's translocation and an *X*-linked enzyme phosphoglycerate kinase (PGK-1; E.C. 2.7.2.3), T16H *Pgk-1^b/+Pgk-1^a*. PGK-1 isozyme expression was monitored by Cello-gel electrophoresis and fluorometric quantitation (Bücher *et al.* 1980). Non-random inactivation in the extra-embryonic tissues is expected (West *et al.* 1977; Harper, Fosten & Monk, 1982) and is indicated by exclusive expression of the *Pgk-1^b* allele on the 16^X chromosome. In the embryonic region 'random' *X*-inactivation is shown by expression of both *Pgk-1* alleles, with a higher PGK-1A activity resulting from the high-expression *Xce^c* locus on the *X* chromosome carrying the *Pgk-1^a* allele (Johnston & Cattnach, 1981). A subsequent loss of the PGK-1A activity occurs, presumably due to selection against cells inactivating autosomal loci on the translocated chromosomes.

2. MATERIALS AND METHODS

(i) *Mice*

Female mice carrying the *X*-autosome translocation, Searle's translocation designated T(X;16)16H (here shortened to T16H; Searle, 1962; Lyon *et al.* 1964) and the *Pgk-1^b* allele, were kindly provided by Dr Sohaila Rastan. The Searle's stock is maintained as illustrated in Table 1A. The Searle's male is sterile and thus females homozygous for the translocated T16H chromosome do not exist. Female mice heterozygous for Searle's translocation and *X*-linked coat markers, tabby

Table 1. Mating schemes for mice carrying Searle's translocation

		(A) Maintenance of Searle's stock*	
Parents	T16H + + / + + Ta +	♂	+ + Blo/Y
Progeny	: + + Blo/ + Ta +	×	: + Ta + / Y
Coat colour	: Blotchy/Stripey		: Tabby
		(B) Production of mice heterozygous for P _{gk} -1 and Searle's translocation*	
Parents	T16H P _{gk} -1 ^b + + / + + P _{gk} -1 ^b Ta +	♂	+ P _{gk} -1 ^a + + / Y
Progeny	: + P _{gk} -1 ^b Ta + / + P _{gk} -1 ^a + +	×	: T16H P _{gk} -1 ^b + + / Y
Coat colour	: Stripey		: Tabby

* Only one of the two possible matings is shown (see Materials and Methods).

Table 2. Expected chromosome constitution of embryos from T16H P_{gk}-1^b / + P_{gk}-1^a × + P_{gk}-1^a / Y matings

Parents	♀		♂		Balanced	Unbalanced	Balanced	Unbalanced
	X	P _{gk} -1 ^a	X	P _{gk} -1 ^a				
Genotype	X ¹⁶	X	16 ^x	16	X	Y	16	16
P _{gk} -1 alleles	—	P _{gk} -1 ^a	P _{gk} -1 ^b	—	P _{gk} -1 ^a	—	—	—
Progeny	♀		♂					
Genotype	X	X	X	X	Y	Y	16 ^x	16
P _{gk} -1 alleles	P _{gk} -1 ^a	P _{gk} -1 ^a	P _{gk} -1 ^a	P _{gk} -1 ^a	P _{gk} -1 ^b	—	—	—
Genotype	X	X	X	X	Y	Y	16	16
P _{gk} -1 alleles	P _{gk} -1 ^a	P _{gk} -1 ^a	P _{gk} -1 ^a	P _{gk} -1 ^a	P _{gk} -1 ^b	—	—	—

For explanation of X¹⁶ and 16^x see Fig. 1.

(*Ta*; T16H + + / + *Ta* +) or blotchy (*Blo*; T16H + + / + + *Blo*) were mated to males + + *Blo*/Y or + *Ta* + /Y, respectively. Females carrying Searle's translocation are phenotypically wild-type and can be distinguished from sisters + + *Blo*/ + *Ta* + which are variegated. Females apparently homozygous for *Ta* or *Blo* indicate cross-over, and are removed from the stock cages.

Females heterozygous for Searle's translocation and *Pgk-1* (T16H*Pgk-1*^b/ + *Pgk-1*^a) were obtained by mating T16H + + / + *Ta* + or T16H + + / + + *Blo* females with males carrying the *Pgk-1*^a allele (+ *Pgk-1*^a/Y) as indicated in Table 1 B. The *Pgk-1*^a mouse stock (kindly provided by Dr 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) by repeated back-crossing. T16H*Pgk-1*^b/ + *Pgk-1*^a females were distinguished from sisters heterozygous for *Ta* or *Blo* by their nonvariegated, wild-type coat. T16H*Pgk-1*^b/ + *Pgk-1*^a females were mated with + *Pgk-1*^a/Y males (Table 2) to generate female embryos of identical genotype to the mother.

(ii) Dissections and sample collections

Matings were set up in a reverse day-night room. Pregnant females were killed between 10.00 and 12.00 h, 6, 7 and 8 days p.c. Embryos were dissected from their decidua into PB 1 (Whittingham & Wales, 1969) containing 0.4 % polyvinylpyrrolidone (PVP) instead of albumin. The extra-embryonic endoderm layer (which has formed yolk-sac endoderm by 8 days) was loosened from the embryonic and extra-embryonic ectoderm regions by digestion in 0.5 % trypsin (Sigma) and 2.5 % pancreatin (BDH) in calcium- and magnesium-free phosphate-buffered saline (PBS) for 10 min at 4 °C (Levak-Švajger, Švajger & Škreb, 1969). Further enzyme digestion was prevented by washing embryos in PB1-PVP containing 10 % foetal calf serum (FCS) at 4 °C. The extra-embryonic endoderm was removed by drawing the embryo through a fine pipette with a diameter just less than the embryo or with electrolytically sharpened tungsten needles.

Six-day embryos were then separated into extra-embryonic ectoderm and epiblast by sucking with a pipette at the junction of the two tissues. A similar procedure was used to separate the extra-embryonic ectoderm from the embryo proper at 7 days. By 8 days the extra-embryonic ectoderm has formed the chorion. The chorion and a sample of the embryo proper were dissected using tungsten needles. Some degenerating 6- and 8-day embryos were collected without dissection. All tissue samples were washed three times in PB1-PVP and collected in 0.5 µl (6 days), 1.0 µl (7 days) or 2.0 µl (8 days) of PB1-PVP in a 5.0 µl Drummond microcap and stored at -70 °C.

In addition, samples of the mother's liver, oocytes and bone marrow were collected. Liver tissue was washed once in PB1-PVP and diluted 1:1 in PB1-PVP in a 2 ml Eppendorf tube. Oocytes were released by tearing the ovary with watchmakers' forceps in a watch glass of PB1-PVP and collected in 1.0 µl of PB1-PVP into a 5 µl Drummond microcap. Bone marrow cells were collected from

the thigh bone in 5 μ l of PB1-PVP in a 10 μ l Drummond microcap. All samples were stored at -70°C .

(iii) *Evaluation of PGK-1 isozyme expression in tissue samples*

Tissue samples were freeze-thawed three times in liquid nitrogen. Liver tissue extract was centrifuged for 3 min at room temperature in a Jobling 320 microfuge and the supernatant diluted 1 in 100 with PBS. All other extracts were centrifuged at 1500 rev/min for 5 min at 4°C in a Beckman model TJ-6 centrifuge. Cellogel electrophoresis and fluorometric quantitation (Bücher *et al.* 1980; McMahon, Foster & Monk, 1981) were employed to determine the PGK-1 isozyme contributions in the supernatants from samples.

3. RESULTS

(i) *Embryo recovery from T16H P gk -1^b/+ P gk -1^a \times + P gk -1^a/Y matings*

In this study all embryos expressing only the *Pgk-1^b* allele in extra-embryonic tissues and both *Pgk-1* alleles in the embryonic region are taken to be balanced T16H *Pgk-1^b/+Pgk-1^a* females. As well as the balanced embryos, a number of chromosomally unbalanced embryos are also expected from segregation of the normal and translocated chromosomes. These are shown in Table 2. Only two of these unbalanced embryos carry both *Pgk-1* alleles (*XX*; 16^X16 and *XY*; 16^X16) and may influence the interpretation of the results. However, both these embryos are partially monosomic for chromosome 16 and are thought to die before implantation (Takagi, 1980). Cross-over and uneven segregation of *X*-chromosome elements may introduce other unbalanced embryos not shown in Table 2. This problem has been dealt with extensively by Takagi (1980) in a study of the genotypes of 75 embryos at 6½, 7½ and 8½ days p.c. which revealed few embryos with chromosomal imbalance. Of these only two would be expected to influence the results reported here. These are female or male embryos which inherit a normal *X*, *Xⁿ*, and a 16^X chromosome from the mother. Most cells from the extra-embryonic and embryonic regions of male embryos of this type show no late-replicating *X* chromosome (Takagi, 1980). In our study we would expect such embryos to show PGK-1A and PGK-1B activity in extra-embryonic tissues as well as in embryonic tissues.

The number of decidua and embryos recovered from matings between T16H *Pgk-1^b/+Pgk-1^a* females and + *Pgk-1^a/Y* males are shown in Table 3. Twenty-three pregnant females were used in this study, the mean number of decidua per female being 6.5. The number of embryos recovered from decidua between 6 and 8 days p.c. was considerably lower, a mean of 3.5 per pregnant female, suggesting early postimplantation death of approximately half the embryos. These probably include chromosomally unbalanced embryos shown in Table 2.

(ii) *PGK-1 expression in tissue samples from T16H Pgk-1^b/+Pgk-1^a mothers*

Adult females heterozygous for Searle's translocation and *Pgk-1* are identified by their non-variegated coat (see Materials and Methods and Table 1). Further verification of the maternal genotype came from examining PGK-1 expression in tissue samples. All 23 expressed exclusively the PGK-1B isozyme in samples of liver

Table 3. *Embryos recovered from T16H Pgk-1^b/+Pgk-1^a × +Pgk-1^a/Y matings*

Day of sacrifice (p.c.)	No. of pregnant females	No. of decidua	No. of embryos recovered
6	13	80	37
7	8	53	31
8	2	17	13
Total	23	150	81

extract. In addition samples of bone marrow cells from females were analysed, and these also expressed only the PGK-1B isozyme. In contrast, oocytes from the 19 females examined expressed both PGK-1A and PGK-1B isozymes as both *X* chromosomes are active. These experiments clearly demonstrate that the females used in this study were indeed heterozygous for Searle's translocation and *Pgk-1*.

(iii) *PGK-1 isozyme expression in embryos from T16H Pgk-1^b/+Pgk-1^a/Y matings*(a) *6-day p.c. embryos*

Six-day p.c. embryos from T16HPgk-1^b/+Pgk-1^a × +Pgk-1^a/Y matings were dissected into visceral endoderm, extra-embryonic ectoderm and epiblast tissue. PGK-1 expression was examined in tissue samples from dissected embryos (Table 4). Of the 37 embryos recovered at 6 days p.c. (Table 3), 28 were successfully dissected to give epiblast and extra-embryonic tissues. For most embryos visceral endoderm and extra-embryonic ectoderm were analysed separately, but as PGK-1 expression was identical in these tissues, the results are shown pooled in Table 4.

Seventeen of the dissected embryos expressed only PGK-1A isozyme in epiblast and extra-embryonic tissues. Ten expressed only PGK-1B isozyme in extra-embryonic tissue but of these only 5 expressed exclusively PGK-1B isozyme in epiblast and are presumably male. The remaining 5 embryos expressed both PGK-1A and PGK-1B isozymes in epiblast (Plate 1, Fig. 1 A, B). One additional embryo showed similar PGK-1A and PGK-1B activity in epiblast but a low level of PGK-1A activity (attributable to contamination) in addition to PGK-1B activity in extra-embryonic ectoderm and visceral endoderm.

PGK-1 isozyme contributions were calculated in 4 of the 6 embryos expressing both PGK-1 isozymes (Table 4). All showed 100% PGK-1B activity in extra-embryonic tissues. In epiblast, two expressed 56% PGK-1B activity, the other two

Table 4. *PGK-1 isozyme expression in 6-day p.c. embryos*

Parents	♀		×	♂	
	T16H <i>Pgk-1^b/+Pgk-1^a</i>			+ <i>Pgk-1^a/Y</i>	
Progeny	T16H <i>Pgk-1^b/+Pgk-1^a</i> ; + <i>Pgk-1^a/+Pgk-1^a</i> ; + <i>Pgk-1^a/Y</i>			T16H <i>Pgk-1^b/Y</i>	
Phenotype	PGK-1B ; PGK-1A			PGK-1A ; PGK-1B	
Extra-embryonic*	PGK-1B ; PGK-1A			PGK-1A ; PGK-1B	
Embryonic	PGK-1A and B ; PGK-1A			PGK-1A ; PBK-1B	
No. observed	6†‡			17 ; 5	
Per cent PGK-1B in embryonic region‡	41.0, 45.0, 56.0, 56.0				

* For most embryos samples of extra-embryonic ectoderm and visceral endoderm were analysed separately. Samples from the same embryo showed identical PGK-1 isozyme expression.

† One embryo showed a low level of PGK-1A expression in the extra-embryonic tissues (not greater than 15.0%) thought to be due to epiblast contamination.

‡ PGK-1 isozyme expression was quantitated fluorometrically in 4 of the 6 samples. The other two samples not quantitated showed equal PGK-1A and PGK-1B isozyme expression as judged by eye.

Table 5. *PGK-1 isozyme expression in 7-day p.c. embryos*

Parents	♀		×	♂	
	T16H <i>Pgk-1^b/+Pgk-1^a</i>			+ <i>Pgk-1^a/Y</i>	
Progeny	T16H <i>Pgk-1^b/+Pgk-1^a</i> ; + <i>Pgk-1^a/+Pgk-1^a</i> ; + <i>Pgk-1^a/Y</i>			T16H <i>Pgk-1^b/Y</i>	
Phenotype	PGK-1B ; PGK-1A			PGK-1A ; PGK-1B	
Extra-embryonic*	PGK-1B ; PGK-1A			PGK-1A ; PGK-1B	
Embryonic	PGK-1A and B ; PGK-1A			PGK-1A ; PGK-1B	
No. observed†	3			18 ; 2	
Per cent PGK-1B in embryonic region	19.0, 39.0, 35.0				

* Samples of visceral endoderm and extra-embryonic ectoderm were analysed separately. Samples from the same showed identical PGK-1 isozyme expression.

† Two additional embryos showed approximately equal PGK-1A and PGK-1B isozyme expression in extra-embryonic regions as well as in embryonic regions (see Table 6).

41.0% and 45.0% PGK-1B activity. The mean value for the four samples was 49.5% PGK-1B activity, not significantly different from 50%. The two other presumed Searle's female embryos not quantitated also showed equal isozyme contributions, as judged by eye, in the epiblast.

(b) 7-day p.c. embryos

A total of 25 7-day p.c. embryos from T16HPgk-1^b/+Pgk-1^a × +Pgk-1^a/Y matings were analysed for PGK-1 expression in embryonic and extra-embryonic regions (Table 5). Eighteen of these expressed only PGK-1A isozyme in all tissue

Table 6. Percentage PGK-1B activity in tissue samples from 7-day p.c. Searle's embryos expressing both PGK-1A and PGK-1B isozymes in the embryonic region

Embryo number	PGK-1B activity		
	Extra-embryonic regions		Embryonic region
	Endoderm	Ectoderm	
1	29.0	49.0	42.0
2	48.0	45.0	43.0
3*	100	100	19.0
4*	100	100	39.0
5*	100	100	35.0

* Embryos 3, 4 and 5 are those shown in Table 5.

samples. Of the remaining embryos, two expressed exclusively PGK-1B isozyme in embryonic and extra-embryonic tissues. Five expressed both PGK-1A and PGK-1B isozymes in embryonic tissue. Table 6 gives the recorded PGK-1 isozyme expression in these heterozygous embryos.

Two of them surprisingly expressed both isozymes in extra-embryonic as well as embryonic regions (Plate 1, fig. 2A, B) showing similar isozyme contributions in both tissues (Table 6; Plate 1, fig. 2A, B). These could be unbalanced karyotypes of the type discussed earlier. The other three are assumed to be Searle's female embryos, and all three showed markedly higher PGK-1A activity in the embryonic region with a mean PGK-1B expression of 31.0%. The rather high variability between embryos can be accounted for by the size of the pool of X-inactivated cells sampled as embryo precursor cells (McMahon *et al.* 1982). When compared with the isozyme contributions in the epiblast of 6-day p.c. embryos from the same mating, the isozyme contributions in the 7-day embryonic region were found to be significantly lower ($t_5 = 2.7$; $P = < 0.05$).

(c) 8-day p.c. embryos

Nine dissected 8-day p.c. embryos were analyzed from T16HPgk-1^b/+Pgk-1^a × Pgk-1^a/Y matings (Table 7). Six showed only PGK-1A expression in extra-embryonic and embryonic tissues. Three embryos expressed only PGK-1B isozyme in extra-embryonic tissues (yolk-sac endoderm and chorion). One of these also expressed exclusively PGK-1B isozyme in embryonic tissue, whereas the other two embryos expressed both PGK-1A and PGK-1B isozymes in embryonic tissue,

but now, in contrast to 7-day p.c. embryos, with PGK-1B activity greater than 50% (Plate 1, fig. 3). The mean PGK-1B activity in the two heterozygous embryonic samples was significantly different from the mean PGK-1B activity at 7 days p.c. ($t_3 = 4.0$, $P < 0.05$).

Table 7. *PGK-1 isozyme expression in 8-day p.c. embryos*

Parents	♀ T16H <i>Pgk-1^b/+Pgk-1^a</i>		×	♂ + <i>Pgk-1^a/Y</i>		
Progeny	T16H <i>Pgk-1^b/+Pgk-1^a</i> ; + <i>Pgk-1^a/+Pgk-1^a</i> ;			+ <i>Pgk-1^a/Y</i> ; T16H <i>Pgk-1^b/Y</i>		
Phenotype						
Extra-embryonic*	PGK-1B	;	PGK-1A	;	PGK-1A ; PGK-1B	
Embryonic	PGK-1A and B	;	PGK-1A	;	PGK-1A ; PGK-1B	
No. observed	2	;	6		;	1
Per cent PGK-1B in embryonic region	64.0, 62.0					

* Samples of yolk-sac endoderm and chorion were analysed. Samples from the same embryo showed identical PGK-1 isozyme expression.

(d) *12½-day p.c. embryos*

PGK-1 isozyme expression in the somatic tissues of gonadally sexed, 12½-day p.c. female embryos from T16H *Pgk-1^b/+Pgk-1^a* × +*Pgk-1^a/Y* matings has been previously reported (Johnston, 1981; McMahon *et al.* 1981). We have analysed three 12½-day p.c. T16H *Pgk-1^b/+Pgk-1^a* females and found no evidence of any PGK-1A isozyme activity. Thus if *Pgk-1^a* expression occurs, the PGK-1A isozyme contribution is less than 1% of the total PGK-1A activity, the limit of the gel sensitivity (McMahon, 1981).

DISCUSSION

Non-random X-chromosome expression observed in female mice heterozygous for Searle's translocation (Lyon *et al.* 1964) may result from either non-random X-inactivation or random X-inactivation followed by cell selection. To distinguish between these alternatives, X-chromosome activity was monitored by the expression of X-linked PGK-1 isozymes in 6-, 7- and 8-day p.c. female embryos heterozygous for Searle's translocation and *Pgk-1*.

Prior to X-inactivation, tissues from female embryos heterozygous for *Pgk-1* would be expected to show equal expression of isozymes as the two isozymic forms of PGK-1 have similar specific activities (West & Chapman, 1978; McMahon, 1981). When X-inactivation occurs in T16H *Pgk-1^b/+Pgk-1^a* female embryos, two possible patterns of PGK-1 isozyme expression in embryonic tissues may result, depending on which of the above alternatives operates.

(a) A higher PGK-1A activity is expected if *X*-inactivation occurs solely under the influence of the *Xce^c* locus. The *Pgk-1^a*-carrying *X* chromosome also carries a high-expression *Xce^c* locus (Johnston & Cattanach, 1981) which biases random *X* inactivation, resulting in a higher proportion of cells (70–80% at 7½ days p.c., Johnston & Cattanach, 1981) with the *Pgk-1^a*-carrying *X* chromosome active.

(b) A higher PGK-1B activity resulting from preferential inactivation of the normal *X* chromosome.

Four 6-day p.c. Searle's female embryos showed approximately equal PGK-1 isozyme expression in the epiblast (mean, 49.5% PGK-1B activity). This suggests that at 6 days p.c. the pattern of PGK-1 isozyme expression does not reflect the *X*-inactivated state in this tissue, supporting the earlier gene-dosage experiments of Monk & Harper (1979). However, it should be borne in mind that enzyme measurements reflect the activity states of the two *X* chromosomes at some earlier stage of development, and these results are not incompatible with the cytogenetic data of Rastan *et al.* (1980), suggesting that *X*-inactivation has occurred by 5½ days p.c.

By 7 days p.c. the PGK-1 isozyme expression in the presumed T16H *Pgk-1^b/+Pgk-1^a* female embryos differed significantly from that measured in the epiblast of embryos at 6 days p.c., with a higher PGK-1A contribution; the mean PGK-1B expression, 31.0%, is similar to the value reported in the embryonic region of 7.5-day p.c. *+Pgk-1^b Xce^a/+Pgk-1^a Xce^c* heterozygotes in which the *Pgk-1^a* allele is paternally inherited (mean PGK-1B expression 28.3%; Johnston & Cattanach, 1981). This result strongly suggests that *X*-inactivation has taken place in the embryonic lineage of T16H *Pgk-1^b/+Pgk-1^a* embryos prior to 7 days p.c., and that *X*-inactivation in Searle's female embryos follows the normal pattern seen in females heterozygous for *Pgk-1* and the *Xce* locus (*Xce^a/Xce^c*).

At 8 days p.c. the situation is reversed and now PGK-1B activity is significantly higher than PGK-1A activity (mean PGK-1B expression 63.0%). This marked switch in the isozyme contributions is most simply explained by selection acting against cells which inactivated the 16^X chromosome. Moreover selection is complete by 12½ days p.c. when the embryo expresses exclusively the *Pgk-1^b* allele; no PGK-1A activity is recorded in somatic tissues at this time (see also Johnston, 1981; McMahon, 1981; McMahon *et al.* 1981).

The mechanism of cell selection is unknown but presumably results from partial monosomy created by inactivation 'spreading' into regions of chromosome 16 from the attached *X*-chromosome segment. Inactivation of autosomal chromatin has been observed in human (Thelen, Abrams & Fisch, 1971; Hagemeyer *et al.* 1977) and mouse (Ohno & Cattanach, 1962; Russell, 1963; Ohno & Lyon, 1965; Cattanach, 1970; Russell & Montgomery, 1970) *X*-autosome translocations. Further, studies on mice heterozygous for certain *X*-autosome translocations have revealed that the proportions of cells with an active translocated *X*-chromosome increases markedly from embryo to adult (Russell & Cacheiro, 1978; Distche *et al.* 1979), also suggesting that cell selection acts against cells with the translocated *X* chromosome inactive.

The data reported here on PGK-1 isozyme expression in Searle's heterozygotes are apparently at variance with the late replication studies of Takagi (1980), in which only a small number of cells ($\sim 4\%$) in the embryonic region (with surrounding visceral endoderm) showed a late replicating 16^X chromosome at $6\frac{1}{2}$ days p.c., suggesting primary non-random X-inactivation in Searle's heterozygotes. Three explanations are offered for the dissimilar findings.

(a) The *Xce^c* locus on the *Pgk-1^a* X chromosome may exert a 'dominant' influence over a normally non-random inactivation event in embryos heterozygous for Searle's translocation.

(b) The 16^X chromosome appears early replicating and therefore active in some cells but is in fact functionally inactive.

(c) Immediately after X-inactivation most cells inactivating the 16^X chromosome cease dividing, therefore few metaphase spreads from $6\frac{1}{2}$ days p.c. embryos in Takagi's study would show a late-replicating 16^X chromosome.

It is not possible at present to distinguish between these alternatives although it is interesting to note, first, that there is no evidence in this study for expression of the *Pgk-1^a* allele on the normal X chromosome in adult bone marrow cells from T16H *Pgk-1^b/+Pgk-1^a* females (the gel system used detects 1% of either PGK-1 isozyme in the presence of the other; McMahon, 1981), although Disteché *et al.* (1981) using a similar technique to Takagi (1980) report that 7% of adult female bone marrow cells have an early replicating and presumably active normal X chromosome. Secondly, cessation of cell division in cells inactivating the 16^X chromosome would lead to extremely rapid changes in cellular proportions in the fast-dividing embryonic tissue, consistent with the dramatic alteration in the pattern of PGK-1 isozyme expression observed between 7 and 8 days p.c.

In conclusion, this study on *Pgk-1* expression in Searle's embryos clearly demonstrates that the preferential expression of the translocated X chromosome is brought about by cell selection following random (excluding the *Xce* effect) X-inactivation and provides a further demonstration (see Snow & Tam, 1979) of the remarkable ability of the mouse embryo to regulate its development when over half the cells, i.e. those inactivating the 16^X chromosome, are presumably lost.

We thank Anne McLaren and Paul Burgoyne for helpful discussions and for their critical reading of the manuscript and T. Bücher and I. Linke for instruction in PGK quantitation.

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EXPLANATION OF PLATE

PLATE 1

Fig. 1. PGK-1 isozyme expression in tissues of two 6-day p.c. female embryos heterozygous for Searle's translocation and *Pgk-1*. (A) PGK-1 isozyme expression in 1, mother's oocytes; 2, mother's thymocytes; 3, epiblast of +*Pgk-1^a*/+*Pgk-1^a* or +*Pgk-1^a*/Y embryo; 4 epiblast of T16H *Pgk-1^b*/+*Pgk-1^a* embryo from the same litter. The PGK-1B isozyme contribution in 4 was calculated to be 56.0%. (B) PGK-1 isozyme expression in 1, mother's oocytes; 2, epiblast of T16H *Pgk-1^b*/+*Pgk-1^a* embryo; 3 and 4, epiblast of +*Pgk-1^a*/+*Pgk-1^a* or +*Pgk-1^a*/Y embryo from the same litter. The PGK-1B isozyme contribution in 2 was calculated to be 45.0%.

Fig. 2. PGK-1 isozyme expression in tissues of a 7-day p.c. unbalanced embryo heterozygous for Searle's translocation and *Pgk-1*. (A) PGK-1 isozyme expression in 1, mother's oocytes; 2, mother's liver; 3, embryonic region of +*Pgk-1^a*/+*Pgk-1^a* or +*Pgk-1^a*/Y embryo; 4, embryonic region of embryo heterozygous for Searle's translocation and *Pgk-1*, presumed to be unbalanced. The PGK-1B isozyme contribution in 4 was calculated to be 43.0%. (B) PGK-1 isozyme expression in 1, visceral endoderm of embryo 3 in Fig. 2A; 2, extra-embryonic ectoderm of embryo 3 in Fig. 2A; 3, visceral endoderm of embryo 4 in Fig. 2A; 4, extra-embryonic ectoderm of embryo 4 in Fig. 2A. The PGK-1B isozyme contribution in 3 and 4 were calculated to be 48.0% and 45.0%, respectively.

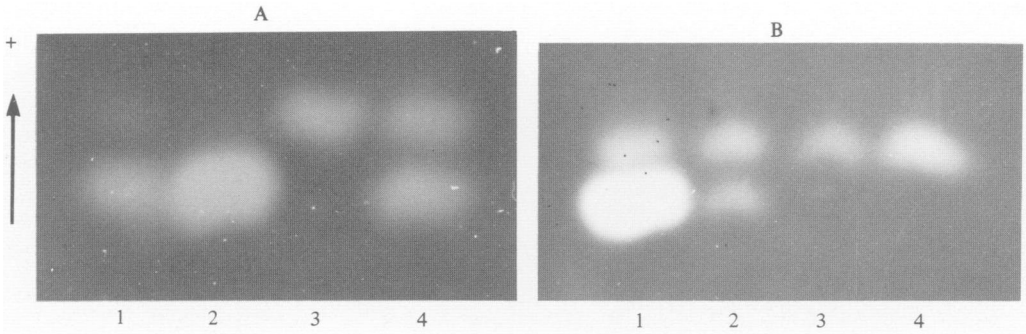


Fig. 1.

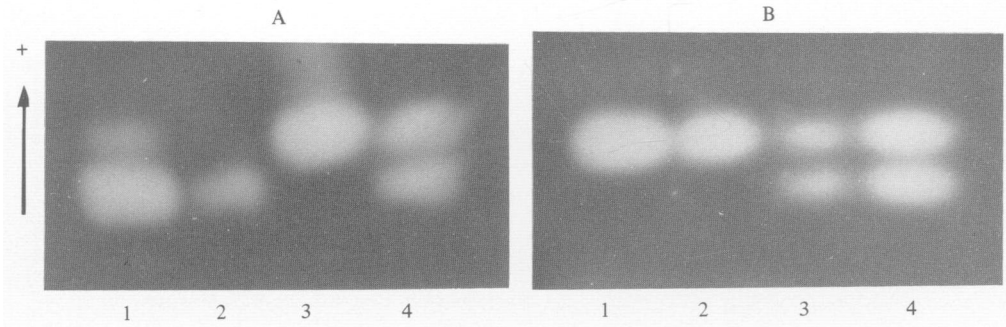


Fig. 2.

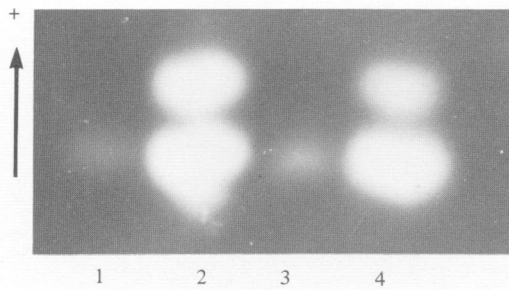


Fig. 3.

Fig. 3. *PGK-1* isozyme expression in tissues of two 8-day p.c. female embryos heterozygous for Searle's translocation and *Pgk-1*. PGK isozyme expression in 1, chorion of T16H *Pgk-1^b/+Pgk-1^a* embryo; 2, embryonic region of embryo in 1; 3, chorion of T16H *Pgk-1^b/+Pgk-1^a* embryo; 4, embryonic region of embryo in 3. The PGK-1B isozyme contributions in 2 and 4 were calculated to be 64.0% and 62.0%, respectively.