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SHORT PAPER

X chromosome activity in female germ cells of mice heterozygous for Searle's translocation T(X;16)16H

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SUMMARY

The expression of X-linked phosphoglycerate kinase (PGK-1) in germ cells from embryos heterozygous for both PGK-1 and Searle's translocation T(X; 16)16H was examined to investigate X chromosome activity during oogenesis. The *Pgk-1*^{b} allele on the translocated X chromosome was the only allele active in somatic cells of all embryos and in germ cells from 12·5 d.p.c. embryos. However, an additional faint band representing *Pgk-1*^{a} activity was observed in germ cells from older embryos (13·5–18·5 d.p.c.) and neonates (1–2 d.p.p.). It is concluded that there is a period when only one X chromosome is active in early female germ cells and that reactivation of the inactive X chromosome takes place just prior to meiotic prophase.

1. INTRODUCTION

The state of X chromosome activity during early oogenesis is still not clear in eutherian mammals. Chromosomal studies have suggested that only one X chromosome is active in migrating primordial germ cells but that reactivation occurs so that both are active in oogonia and oocytes (Ohno, 1963). Electrophoresis of ovarian extracts from human foetuses of different ages, heterozygous for the X-linked dimeric enzyme, glucose-6-phosphate dehydrogenase (*EC*.1.1.1.49: G6PD) has also been used to investigate X chromosome activity. Gartler *et al.* (1975) found that a heterodimer band, indicative of two active X chromosomes, was not present in ovary preparations containing only oogonia, but was present in preparations with some meiotic cells. They suggested that reactivation of the second X took place about the time of entry of germ cells into meiosis. Migeon & Jelalian (1977) in a similar study found heterodimer formation not only in ovarian preparations containing oocytes but also in an essentially premeiotic preparation from an 8 week old foetus. They concluded that, although their data failed to differentiate between the escape of germ cells from inactivation and reactivation of the second X, it excluded reactivation coincident with the onset of meiosis.

A similar approach using mice could possibly clarify the situation because the mouse gonad shows less overlap of germ cell stages than does the human gonad (Borum, 1961; Baker, 1963) and it is possible to obtain preparations with a significantly higher percentage of germ cells (Gartler *et al.* 1975). However, no G6PD polymorphism has yet been reported in the laboratory mouse.

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A polymorphism for X-linked phosphoglycerate kinase (*EC*.2.7.2.3; PGK) does occur in the mouse (Neilsen & Chapman, 1977) but this enzyme is a monomer. Unlike studies with G6PD it is therefore not possible to distinguish between activity of both alleles in the same cell and activity of either allele in different cells because both situations will give rise to a double banded phenotype in heterozygous individuals. An indirect method of examining X chromosome activity in germ cells of the mouse has therefore been devised using mice heterozygous for PGK and Searle's translocation T(X;16)16H. The rationale for this is that studies on mice heterozygous for this translocation and other X-linked loci have shown that only alleles on the translocated X chromosome are active (Lyon *et al.* 1964). If it can be established that the PGK locus behaves in a similar manner in somatic cells then it should be possible to examine X chromosome activity during oogenesis. Germ cells with only one X chromosome active would express a single banded phenotype with only the allele on the translocated X chromosome active. The appearance of a double banded phenotype would indicate that both alleles and presumably both X chromosomes are active in the same germ cell.

2. MATERIALS AND METHODS

Animals The variant *Pgk-1*^{a} allele originated from feral *Mus. m. musculus* captured in Denmark (Neilsen & Chapman, 1977). Mice with this allele were subsequently back-crossed to the C3H/HeHa strain (West and Chapman, 1978) and a breeding nucleus was kindly provided by Dr J. D. West of the Zoology School, Oxford University. The T(X;16)16H translocation commonly known as Searle's translocation, is a reciprocal translocation between the X chromosome and chromosome 16 (Eicher *et al.* 1972), which arose and is maintained at Harwell.

In order to obtain *T16H Pgk-1*^{b}/ + *Pgk-1*^{a} heterozygotes, crosses were set up between *T16H Pgk-1*^{b}/ + *Pgk-1*^{b} or *T16H Pgk-1*^{b}/ + *Pgk-1*^{a} females X + *Pgk-1*^{a}/Y males.

Dissection and sample preparation

Conceptuses ranging in age from 12.5d to 18.5d were removed from the uterus and embryos dissected free of extra-embryonic membranes. The sex of females less than 13·5 d.p.c. was confirmed by sex chromatin staining of the amnion with aceto-orcein (Vickers, 1967), and older embryos were sexed morphologically. The gonads were removed and placed in phosphate buffered saline (PBS). Germ cells were collected following the methods of Heath (1978) and transferred to a microcentrifuge tube. After centrifugation at 1000 rev/min for 5 min, the PBS was reduced in volume to approximately 2 μl and the cells lysed by freezing and thawing with liquid nitrogen. For study of somatic tissues embryos were homogenized in gel buffer, 3 % (w/v) and then centrifuged at 3000 rev/min for 10 min prior to electrophoresis.

Electrophoresis

Samples were applied onto Cellogel strips (7·8 × 15 cm) and electrophoresis was carried out in the tris-EDTA-citric acid (pH = 7·5) buffer of Rattazzi *et al.* (1967), for 2 h with a voltage gradient of 14 V cm^{-1}. The gels were stained as described by Cooper *et al.* (1971) and examined under u.v. light at 365 nm. When staining intensity was judged to have reached its optimum the filter paper overlay was removed and the gels were placed in a mixture of 20 ml staining buffer, 0·5 ml phenazine methosulphate (3 mg/ml), 0·5 ml MTT tetrazolium (5 mg/ml) before being thoroughly washed in water. The gels were then photographed.

Cytology

Air dried preparations of germ cells were made after removing the PBS and adding fixative (3 parts methanol:1 part glacial acetic acid). The slides were stained with toluidine blue as described by Breckon and Evans (1969).

Table 1. *Phosphoglycerate kinase (PGK–1) activity in somatic and germ cells of* T16H Pgk–1^b/+ Pgk–1^a *heterozygotes*

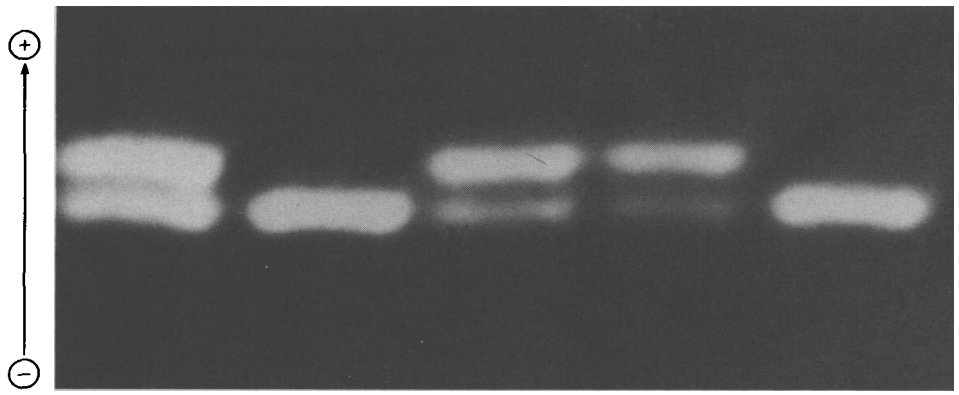
		Somatic cells		Germ cells	
Age	N	PGK-1A	PGK-1B	PGK-1A	PGK-1B
12·5 d.p.c.	5	–	+	–	+
13·5 d.p.c.	2	–	+	+	+
14·5 d.p.c.	2	–	+	+	+
15·5 d.p.c.	2	–	+	+	+
16·5 d.p.c.	2	–	+	+	+
17·5 d.p.c.	2	–	+	+	+
18·5 d.p.c.	1	–	+	+	+
1 d.p.p.	1	–	+	+	+
2 d.p.p.	3	–	+	+	+

3. RESULTS

The results are summarized in Table 1 and examples of the electrophoretic phenotypes are shown in Plate 1. The PGK-1 phenotypes of somatic cell homogenates from embryos produced by mating female translocation heterozygotes homozygous for *Pgk-1^b* with males hemizygous for *Pgk-1^a* are shown in Plate 1, fig. 1. Both translocation heterozygotes and individuals with normal X chromosomes are expected from this mating. PGK-1B is the only detectable allozyme in homogenates of a 12·5 d.p.c. embryo (channel 2) and one 13·5 d.p.c. embryo (channel 5). The most plausible explanation for this observation is that these embryos are translocation heterozygotes and that only the translocated X chromosome carrying the *Pgk-1^b* allele is active in the somatic cells of these individuals. The remaining embryos show double banded phenotypes (PGK-1AB) and are assumed to have normal X chromosomes. The reason for the greater activity of the PGK-1A allozyme in embryos (Channels 1, 3, 4) is that the *Pgk-1^a* marked X chromosome has a particular *Xce* allele (*Xce^c*) which increases the probability of the X chromosome carrying it being active (Johnston and Cattanach, 1979). Table 1 shows that there was no evidence of PGK-1A allozyme activity in somatic tissues from any of the different aged embryos or newborn *T16H Pgk-1^b/+ Pgk-1^a* heterozygotes. Homogenates of liver, lung, heart, muscle, kidney, spleen and brain from adult heterozygotes similarly expressed only the PGK-1B allozyme.

Cytological examination of germ cell preparations from gonads of 12·5 d.p.c. females showed an abundance of oogonia, many of which were undergoing mitosis. At 13·5 d.p.c. oogonia were still proliferating mitotically but between 10–20 % had progressed to the preleptotene condensation stage. At 14·5 d.p.c., the majority of germ cells had entered meiosis with approximately equal numbers in leptotene and zygotene. Pachytene was the most common stage from 15·5 d.p.c. to birth. Many of the cells underwent atresia during this period. The proportion of germ cells to somatic cells in the preparations studied was high (85–90 %) in 12·5–16·5 d.p.c. embryos but lower (60–70 %) for late term embryos and new born mice.

Electrophoresis of germ cells from 12·5 d.p.c. embryos showed only PGK-1B expression (Plate 1, fig. 2A). However, germ cells from 13·5 d.p.c. and older embryos showed an additional faint PGK-1A band (Plate 1, fig. 2B, C, and D). The activity of this allozyme, relative to that of PGK-1B remained at a low level in germ cells from females 13·5 d.p.c. to 2 d.p.p.



	1	2	3	4	5
Age	12·5 d.p.c.	12·5 d.p.c.	13·5 d.p.c.	13·5 d.p.c.	13·5 d.p.c.
Genotype	$\frac{+\,b}{+\,a}$	$\frac{T\,b}{+\,a}$	$\frac{+\,b}{+\,a}$	$\frac{+\,b}{+\,a}$	$\frac{T\,b}{+\,a}$

Fig. 1. PGK-1 electrophoresis patterns of somatic cell homogenates from 12·5 and 13·5 d.p.c. embryos. Inferred genotypes are given below the channel number. T = *T16H* X chromosome; + = normal X chromosome; a = *Pgk-1*^{a}; b = *Pgk-1*^{b}.

4. DISCUSSION

These results show that in somatic cells, the expression of the *Pgk-1* locus when associated with Searle's translocation is similar to that of other sex linked loci such as tabby (Ta), blotchy (Blo) and bent tail (Bn) (Lyon *et al.* 1964). It therefore appears that all X linked alleles on the translocated X chromosome are completely dominant over those on the normal X chromosome in somatic tissues.

In germ cells, initially only the *Pgk-1* allele on the translocated X chromosome is active at 12·5 d.p.c. The germ cells at this stage are behaving like somatic cells and have only one X chromosome active. Thus although controversy still exists regarding X chromosome activity in germ cells of human foetuses it is apparent from these results that there is a period during oogenesis when only one X chromosome is active in female mouse germ cells. At 13·5 d.p.c., both alleles are active and presumably reactivation of the normal X chromosome has occurred resulting in both X chromosomes being active in the same germ cell. It is difficult to explain why the activity of the PGK-1A allozyme remained low and did not reach a similar level of activity to that of the PGK-1B allozyme, at least in older embryos or new born mice. It is unlikely that somatic cell contamination was responsible during the embryonic stages (13·5–15·5 d.p.c.) because of the high proportion of germ cells present. However, it might have been a contributing factor in germ cell preparations of older embryos and new born mice, where somatic cell contamination was higher and degeneration of pachytene oocytes was common. Another possible explanation is that during early stages of meiosis the synthesis of both allozymes is relatively low

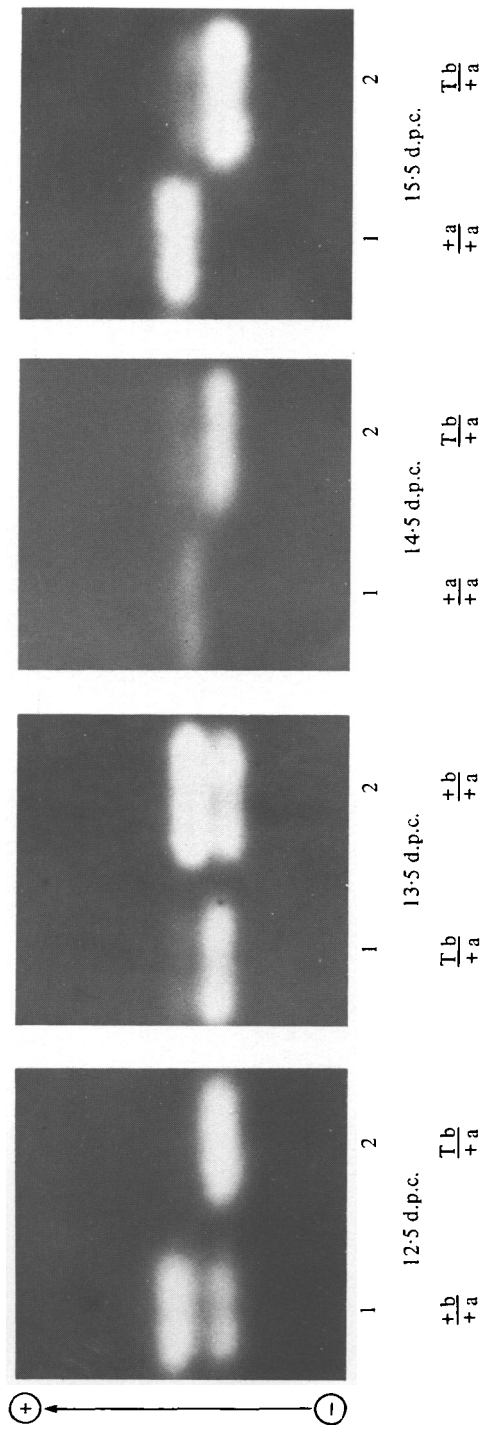


Fig. 2. PGK-1 electrophoresis patterns of germ cell preparations from 12·5 d.p.c. (A), 13·5 d.p.c. (B), 14.5 d.p.c. (C), and 15.5 d.p.c. (D) embryos. Inferred genotypes are given below the channel number.

and that the major contribution to enzyme activity had been made by premeiotic oogonia which were synthesizing only PGK-1B allozyme. Unfortunately, no information is available on the half life or rates of transcription of PGK in germ cells. However, there is evidence which suggests RNA synthesis is relatively low during early stages of meiosis in the mouse (Bakken and McClanahan, 1979). They found that H^3 uridine incorporation was abundant in premeiotoic oogonial nuclei, less so in leptotene and zygotene, little or none in pachytene but that it did increase again markedly in diplotene nuclei. Quantitative studies on G6PD activity in germ cells of XX and XO mice by Andina (1978) also indicate that synthesis of enzyme from the 'reactivated' allele may be low during early stages of meiosis. He found that the activity of G6PD was similar in both groups during early meiotic stages but an XX/XO ratio of 2:1 was approached in germ cells of new born mice and he concluded that the data were compatible with X chromosome reactivation at the time of entry into meiosis.

A recent study on quantitative levels of the X linked enzyme hypoxanthine phosphoribosyl transferase (HPRT, *EC*.2.4.2.8) by Monk and McLaren (personal communication) suggested that only one X chromosome was expressed in female germ cells at some stage prior to the onset of meiosis, although they were unable to say when reactivation of the second X chromosome occurred. Similarly, it is not possible to pinpoint the precise timing of reactivation from this study. However, the expression of the *Pgk-1*^{a} allele in premeiotic germ cells of 13·5 d.p.c. embryos indicates that reactivation takes place earlier than the onset of meiosis. Although the exact timing of reactivation is yet to be resolved these data provide additional proof from another sex linked locus that germ cells of mice do not escape X inactivation.

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