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GPI expression in female germ cells of the mouse

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SUMMARY

The genetically determined oocyte-specific expression of glucose-phosphate isomerase activity in the mouse is first apparent at 6 to 7 days after birth, and occurs in *XO* as well as in *XX* oocytes. The regulator locus that controls oocyte-specific expression shows the same linkage relations as the structural gene, suggesting that both form part of a *Gpi-1* gene complex.

1. INTRODUCTION

Glucosephosphate isomerase (GPI; EC 5.3.1.9) occurs in the mouse in two electrophoretically distinct forms, GPI-1A and GPI-1B (De Lorenzo & Ruddle, 1969), coded by alleles $Gpi\text{-}1^a$ and $Gpi\text{-}1^b$. The enzyme is a dimer, and heterozygotes ($Gpi\text{-}1^a/Gpi\text{-}1^b$) yield a 3-banded electrophoretic pattern, corresponding to enzyme molecules of constitution AA, AB and BB. In most tissues of the body the intensity of the bands shows a 1:2:1 ratio, as would be expected if the A and B subunits are approximately equal in both activity and number, and associate randomly. However, Peterson & Wong (1978) studied GPI expression in unfertilized eggs of the inbred mouse strains DBA ($Gpi\text{-}1^{a/a}$), C57BL ($Gpi\text{-}1^{b/b}$) and LP ($Gpi\text{-}1^{b/b}$), and found that GPI activity was some four times higher in C57BL and LP eggs than in DBA eggs. Eggs of F_1 hybrids between C57BL and DBA had intermediate activity levels and an electrophoretic pattern strongly skewed in favour of GPI-1B, showing that the activity was *cis*-regulated. From F_1 red blood cells, the ratio of the three bands was the expected 1:2:1, but from eggs, the ratio was 3:30:67.

Peterson & Wong (1978) examined 65 eggs from heterozygous females of the backcross generation (C57BL × DBA)F_1 × DBA, and found that the electrophoretic pattern in each was still skewed in favour of GPI-1B, i.e. no recombination had occurred between the electrophoretic and the activity variant. They concluded that there exists a regulator locus for oocyte regulation of GPI-1, named by them *Org*, closely linked to *Gpi-1*.

This appears to be the first example of tissue-specific genetic variation in enzyme activity affecting the mammalian egg. We have looked to see at what stage in the ontogeny of the female germ line it is first expressed.

2. MATERIALS AND METHODS

(i) *Mice*

Two *Gpi-1*$^{b/b}$ stocks were used, C57BL and SWR, and two *Gpi-1*$^{a/a}$ stocks, A2G and a multiple recessive (MR) strain, homozygous for *a*, *b*, *d*, *p*, *c*^{ch}, *vt*, *se* and *wv*-2.

(ii) *Sample preparation*

Foetal germ cells were obtained by mashing the gonads after they had been freed of adjacent tissue under a dissecting microscope. The cells released were transferred to saline in an agar-coated dish, and the germ cells separated from the contaminating somatic cells manually, using a fine glass pipette. The large round germ cells were easy to recognize: their identity was confirmed initially by testing for alkaline phosphatase activity (Brinster & Harstad, 1977); 50–100 of these cells constituted a single sample. Oocytes of mice 4–10 days p.p. were obtained according to the method of Mangia & Epstein (1975). Oocytes of adults were obtained by removing the ovaries to a dish of phosphate-buffered saline (PBS) and opening antrum-containing follicles with fine forceps. Oocytes were freed of follicle cells by repeated sucking into a pipette with a diameter sufficiently narrow to strip off any adhering cells; 20–60 oocytes could be obtained from a single female, and these were pooled for electrophoretic analysis. Measurements of oocytes were made using an eyepiece graticule on a dissecting microscope, and samples of oocytes and foetal germ cells were taken up in roughly 3–4 μl PBS and pipetted into 10 μl microcapillary tubes. These were flame-sealed at both ends and frozen. Histological preparations of ovaries were made by fixing them in Bouin's fluid, embedding in wax, sectioning at 8 μm, and staining in haematoxylin and eosin.

(iii) *Electrophoresis*

Electrophoresis was normally done on 'Cellogel' cellulose acetate strips 2·5 × 17 cm in size. For a general description of the 'Cellogel' system and its use see Meera Khan (1971). The bridge width was 11 cm, and samples were applied to the strips midway between the bridge shoulders. The samples were removed from their microcaps and either pipetted directly on to the gel surface, or transferred to a glass slide and picked up on a 'Cellogel' micro-applicator, which was then applied to the gel. The latter method involved some loss of sample, but the band resolution was better. The bridge buffer was 0·1 M-Tris-glycine (pH 8·5) and gels were normally run at 200 V for 2 h at room temperature. In this system the GPI moves towards the cathode, with the A form moving more slowly than the B. The stain used consisted of 8 mg fructose 6-phosphate, 8 mg $MgCl_2$, 4 mg NADP, 4 mg nitro blue tetrazolium, 2 mg phenazine methosulphate, and 10 units of glucose-6-phosphate dehydrogenase (all obtained from Sigma) in 2 ml 0·05 M-Tris-HCl buffer, pH 8·0, and mixed immediately before use with 4 ml molten 2·5% agar made up in the same buffer. The gel strips were covered with the stain and incubated at 37 °C in the dark. GPI bands could be seen within 20 min. This 'Cellogel'

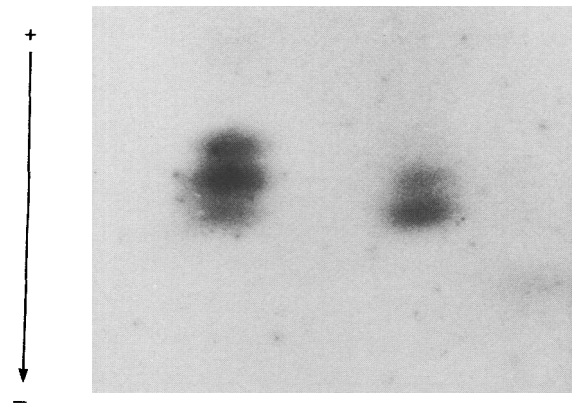

Fig. 1. Electropherogram of GPI from blood (*a*) and oocytes (*b*) of an F_1 female derived from a C57BL × MR cross. ('Cellogel' system)

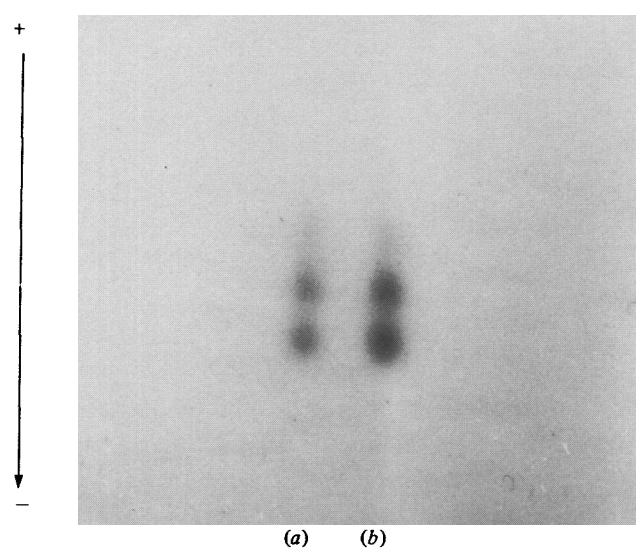

Fig. 2. Electropherogram of GPI from oocytes of *XX* (*a*) and *XO* (*b*) females, both from a cross of a GPI-$1^{b/b}$ *XO* female and an MR male. ('Titan III' gel plate)

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system, though providing adequate results with most of our samples, was found not to be satisfactory when samples of fewer than 3 or 4 oocytes were run. In the latter part of this study some samples were run on Titan III cellulose acetate gel plates (from Helena Laboratories) which resulted in improved resolution of small samples. In this case the bridge buffer was 0·09 M-Tris-glycine (pH 8·5) and gels were run at 200 V for 1 h. They were stained according to the method of Eicher & Washburn (1978).

3. RESULTS

C57BL mice (*Gpi-1*$^{b/b}$) were crossed with either A2G or MR mice (both *Gpi-1*$^{a/a}$). Blood and oocytes from F_1 female progeny of the first cross both gave a symmetrical electrophoresis pattern; from the second cross, blood gave a symmetrical pattern but the oocyte pattern was skewed in favour of GPI-1B (Plate 1, Fig. 1). This established that, in the terminology of Peterson & Wong (1978), our

Table 1. *GPI-1 activity of unfertilized eggs of C57BL and MR mice*

Strain	Number of assays	Number of eggs	GPI activity (nm NADPH/egg/h) Mean ± S.E.
C57BL	6	60	1·44 ± 0·18
MR	9	90	0·55 ± 0·05

Table 2. *Change in pattern of GPI expression in oocytes from mice of different ages*

(Each group consisted of female sibs from a single pregnant female.)

Days of of age	Approximate range of oocyte size (μm)	Relative intensity of homopolymer bands	Histology of largest follicles in other females of same age
14½ p.c.		A = B	
16½ p.c.		A = B	
17½ p.c.		A = B	
4 p.p.	30–60	A = B	
5 p.p.	30–60	A = B	1-layered
6 p.p.	30–60	A < B	
7 p.p.	40–80	A < B	2- and 3-layered
8 p.p.	40–80	A < B	2- and 3-layered
9 p.p.	40–80	A < B	
10 p.p.	40–80	A absent	

C57BL and A2G strains were both *Org*$^{high/high}$, while our MR strain was *Org*$^{low/low}$. The degree of skewing was such that the BB and AB bands were of approximately equal intensity, and only a very faint AA band was seen. This is consistent with a twofold difference in activity or amount between the A subunits from the MR oocytes and the B subunits from the C57BL oocytes. Biochemical assays of GPI

Table 3. *'Linkage' test carried out by Peterson & Wong (1978). No recombinants between* Org *and* Gpi-*1 observed; if* Org *is indeed a separate regulator locus, it must be very closely linked to* Gpi-*1*

Parental	(DBA) $\frac{Org^{low}Gpi\text{-}1^{a}}{Org^{low}Gpi\text{-}1^{a}}$ × (C57BL) $\frac{Org^{high}Gpi\text{-}1^{b}}{Org^{high}Gpi\text{-}1^{b}}$			
F_1 progeny	$\frac{Org^{low}Gpi\text{-}1^{a}}{Org^{high}Gpi\text{-}1^{b}}$ × $\frac{Org^{high}Gpi\text{-}1^{b}}{Org^{high}Gpi\text{-}1^{b}}$			
Back-cross progeny	$\frac{Org^{low}Gpi\text{-}1^{a}}{Org^{high}Gpi\text{-}1^{b}}$	$\frac{Org^{high}Gpi\text{-}1^{a}}{Org^{high}Gpi\text{-}1^{b}}$	$\frac{Org^{low}Gpi\text{-}1^{b}}{Org^{high}Gpi\text{-}1^{b}}$	$\frac{Org^{high}Gpi\text{-}1^{b}}{Org^{high}Gpi\text{-}1^{b}}$
	(Non-recombinant)	(Recombinant)	(Recombinant)	(Non-recombinant)
Progeny observed	65	0	—	—

Table 4. *'Linkage' test between* Org *and* c. *The test estimates crossing over between these two loci, in mice homozygous for* Gpi-1^{a}, *but the results of recombination can only be scored in heterozygous* Gpi-$1^{a/b}$ *animals. The estimated map distance, 22 map units, does not differ significantly from the value for the* c–Gpi-^{1} *interval (29 map units)*

Parental	(MR) $\frac{Org^{low}Gpi\text{-}1^{a}\ c^{ch}}{Org^{low}Gpi\text{-}1^{a}\ c^{ch}}$ × (A2G) $\frac{Org^{high}Gpi\text{-}1^{a}\ c}{Org^{high}Gpi\text{-}1^{a}\ c}$			
F_1 progeny	$\frac{Org^{low}Gpi\text{-}1^{a}\ c^{ch}}{Org^{high}Gpi\text{-}1^{a}\ c}$ × (SWR) $\frac{Org^{high}Gpi\text{-}1^{b}\ c}{Org^{high}Gpi\text{-}1^{b}\ c}$			
	$\frac{Org^{low}Gpi\text{-}1^{a}\ c^{ch}}{Org^{high}Gpi\text{-}1^{b}\ c}$	$\frac{Org^{high}Gpi\text{-}1^{a}\ c^{ch}}{Org^{high}Gpi\text{-}1^{b}\ c}$	$\frac{Org^{low}Gpi\text{-}1^{a}\ c}{Org^{high}Gpi\text{-}1^{b}\ c}$	$\frac{Org^{high}Gpi\text{-}1^{a}\ c}{Org^{high}Gpi\text{-}1^{b}\ c}$
	(Non-recombinant)	(Recombinant)	(Recombinant)	(Non-recombinant)
Progeny observed	16	5	4	16
Progeny expected if map distance = 29 units	14·5	6·0	6·0	14·5

activity in oocytes of C57BL and MR females, using the method of Brinster (1973), confirmed that the level of activity in MR oocytes was of the order of half that found in C57BL oocytes (Table 1).

No maternal effect was apparent, in that the degree of skewing of the electrophoretic pattern from heterozygous oocytes was similar whether the oocytes were obtained from (MR♀ × C57BL♂) F_1 or from (C57BL♀ × MR♂) F_1 females.

To find out at what stage the skewed pattern first appeared, germ cells were recovered from (C57BL × MR) females at different ages, both before and after birth. Table 2 shows that the germ cells collected from the foetal ovaries gave a symmetrical pattern, and the skewing appeared abruptly, between 6 and 9 days after birth. Histological examination revealed that this was the age at which, in mice of this genotype, the first growing oocytes appeared in the ovary. After this age, all oocyte samples gave a skewed pattern.

Oocytes are known to differ from all somatic tissues of female mammals in having both *X* chromosomes active. To see whether the oocyte-specific regulation of GPI-1 activity required the presence of two active *X* chromosomes, a $Gpi\text{-}1^{b/b}$ *XO* female carrying the *X* chromosome marker *Harlequin* (*Hq*) was mated with an MR male. $X^{+}X^{Hq}$ and $X^{+}O$ female progeny were distinguished by their coat patterns, and oocytes from both were recovered and tested. The electrophoretic pattern proved to be similarly skewed for both *XO* and *XX* oocytes (Plate 1, Fig. 2).

The conclusion of Peterson & Wong (1978) that oocyte expression of *Gpi-1* was controlled by a locus within or closely linked to the *Gpi-1* locus depended on finding no recombinants when a low-activity $Gpi\text{-}1^{a}$ allele was paired with a high-activity $Gpi\text{-}1^{b}$ allele (Table 3). To see whether the linkage would hold when two $Gpi\text{-}1^{a}$ alleles were paired, one low and the other high, the test summarized in Table 4 was carried out, making use of the known linkage between the *Gpi-1* and the *c* locus. The proportion of recombinants between *c* and *Org* (Table 4) is consistent with the conclusion that the regulator locus is closely linked to or within the *Gpi-1* structural gene.

4. DISCUSSION

Org is a 'temporal' gene (Paigen, 1979) in the sense that it is involved in determining the programme for GPI concentration during development; mutations at this locus would thus affect the developmental sequence of expression and relative tissue distribution of GPI. Similar regulatory systems have been detected in other organisms, such as maize and *Drosophila* (reviewed by Paigen, 1979). In certain strains of *Drosophila*, for example, amylase appears in both the posterior and anterior segments of the midgut at the time that the adult flies emerge, but in other strains amylase synthesis is not initiated in the posterior midgut until much later (Abraham & Doane, 1978). The time of appearance of the enzyme is controlled by a locus, *Map*, that undergoes recombination with the structural gene and that acts *trans*.

Temporal genes that are more tightly associated with their structural locus mostly act *cis*, as does *Org*. Failure to detect recombination between a temporal

and a structural gene could indicate either that the temporal is situated inside the structural locus, or that the two are very closely linked. Paigen (1979) favours the second possibility, since for many such systems there is no strain concordance between alleles at the two loci. The issue remains unresolved for *Org*, though it seems that here too there is no strain concordance: Peterson (personal communication) has found both *Gpi-1*^{a} and *Gpi-1*^{b} associated with varying degrees of expression at the regulator locus.

If *Org* differentially represses or activates the expression of *Gpi-1* in oocytes, what is the signal to which the regulator gene is responding? In the normal female germ line, the second *X* chromosome undergoes reactivation shortly before the onset of meiosis (Monk & McLaren, 1981), but our results on *XO* oocytes show that this does not affect the regulation of *Gpi-1*. It also seems unlikely that the onset of meiosis itself acts as a signal, since in female mice meiosis begins nearly a week before birth, and we have found no indication of the characteristic oocyte GPI-1 pattern until several days after birth.

Indeed, in the strain combination that we examined, the similarity in timing between the initiation of *Org* activity and the start of oocyte growth suggests that these two events may be related. The activities of glucose-6-phosphate dehydrogenase and lactate dehydrogenase also begin to show a rapid rise at about this time (Mangia & Epstein, 1975). The growing phase of the oocyte is normally heralded by a change in both shape and number of the surrounding follicle cells (Lintern-Moore & Moore, 1979); however, under exceptional circumstances oocyte growth can occur with little or no development of the follicle (McLaren, 1980). Whether the signal for the regulation of *Gpi-1* emanates from the follicle cells, or whether it is part of the endogenous developmental programme of the oocyte has yet to be elucidated.

Since the regulator gene and the structural gene appear to form part of a single gene complex, the symbol *Org* is not in line with the rules laid down by the Committee on Standardized Genetic Nomenclature for Mice (1979). We suggest that the regulator gene be regarded as a temporal gene expressed in the oocyte, and hence designated *Gpi-1t*°.

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