

## Quantification of the transition from oocyte-coded to embryo-coded glucose phosphate isomerase in mouse embryos

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### SUMMARY

A quantitative electrophoretic analysis of glucose phosphate isomerase (GPI-1) allozymes produced by heterozygous *Gpi-1s<sup>a</sup>/Gpi-1s<sup>b</sup>* mouse embryos has enabled us to estimate separately the contributions of GPI-1 enzyme that were (1) oocyte coded, (2) encoded by the embryonic, maternally derived *Gpi-1s<sup>a</sup>* allele and (3) encoded by the embryonic, paternally derived *Gpi-1s<sup>b</sup>* allele. The oocyte-coded GPI-1 activity is stable until 2½ days and then declines and is exhausted by 5½ to 6½ days *post coitum* (*p.c.*). The maternally and paternally derived *Gpi-1s* alleles are probably usually activated synchronously but several possible exceptions were observed. This activation was first detected in 2½-day embryos. Total GPI-1 activity falls to a minimum around 3½ to 4½ days, even though embryonic gene expression has already begun. The profile of oocyte-coded GPI-1 activity is consistent with the suggestion (Harper & Monk, 1983) that there is a mechanism for the removal of oocyte-coded gene products at around 2½ days *p.c.* The method of analysis described is applicable to other dimeric enzymes with electrophoretic variants.

### INTRODUCTION

During the first few days of mammalian development the gene products produced by the diploid oocyte are replaced by new gene products synthesized by the embryonic genome. Some embryonic gene products can be detected in the 2-cell mouse embryo at 1½ days *post coitum* (*p.c.*) while other products are detectable at later stages (see Johnson, 1981; Magnuson & Eptstein, 1981 for reviews). A number of studies have shown that the embryo-coded structural gene *Gpi-1s*, that encodes the dimeric enzyme glucose phosphate isomerase, (GPI-1; E.C. 5.3.1.9) is expressed in preimplantation mouse embryos (Chapman, Whitten & Ruddle, 1971; Brinster, 1973; West & Green, 1983; Duboule & Bürki, 1985; Gilbert & Solter, 1985).

The embryo-coded GPI-1 enzyme is usually first detectable between 2½ and 3½ days *p.c.* and the oocyte-coded enzyme has been shown to persist until 5½ or 6½

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days *p.c.* However, the kinetics of the decline of oocyte-coded GPI-1 has not been investigated.

We now report the results of a novel analysis of a quantitative electrophoresis study that has enabled us to estimate separately, for the first time, the proportions of GPI-1 enzyme that are (1) oocyte-coded, (2) encoded by the maternally derived *Gpi-1s<sup>a</sup>* allele in the embryo and (3) encoded by the paternally derived *Gpi-1s<sup>b</sup>* allele in the embryo. This analysis provides a quantitative description of the transition from oocyte-coded to embryo-coded enzyme in early embryos and indicates that the decline in oocyte-coded enzyme begins at 2½ days *p.c.* The study also confirms that the two embryo-coded *Gpi-1s* alleles are usually activated synchronously and are first detectable at 2½ days *p.c.*

## MATERIALS AND METHODS

### (A) Mice

The inbred strains of mice 129/Sv-*Sl<sup>1</sup>-CP* and C57BL/Ola (abbreviated to 129 and B respectively) were maintained in the Sir William Dunn School of Pathology, Oxford and the Centre for Reproductive Biology, Edinburgh. Conventional light/dark cycles in Oxford (dark period 7 p.m. to 7 a.m.) and Edinburgh (dark period 7.30 p.m. to 5 a.m.) were used to induce mating close to midnight. In addition, some mice were housed in Oxford under a different lighting regime where the dark period was between 2 p.m. and midnight. Under these conditions mating is assumed to occur most commonly at around 7 p.m. (the midpoint of the dark cycle). Heterozygous *Gpi-1s<sup>a</sup>/Gpi-1s<sup>b</sup>* mice and embryos were produced by crossing 129 strain females (*Gpi-1s<sup>a</sup>/Gpi-1s<sup>a</sup>*) with C57BL/Ola strain males (*Gpi-1s<sup>b</sup>/Gpi-1s<sup>b</sup>*).

### (B) Collection of embryos and eggs

Preimplantation embryos were collected at approximately 9–11 a.m. on various days following the detection of a vaginal plug. Most of the embryos were produced by natural matings and the time of mating was taken to be the midpoint of the dark period prior to the detection of the vaginal plug. Embryos collected during the morning when the vaginal plug was first detected were designated 0.4 days *post coitum* (*p.c.*) for mice housed under conventional lighting regimes and 0.6 days *p.c.* for those housed with a dark period from 2 p.m. to midnight.

The unfertilized eggs and some preimplantation embryos (marked in the tables) were produced by induced ovulation. Female mice were induced to ovulate with intraperitoneal injections of 5 i.u. pregnant mares' serum gonadotrophin (PMS) followed 48 h later by 5 i.u. human chorionic gonadotrophin (HCG). These injections were given at 11 a.m.–12 noon and the time of mating is taken to be approximately midnight, when ovulation is expected to begin. Embryos recovered on the day following the HCG injection were designated 0.4 days *p.c.*

Preimplantation embryos were flushed from the reproductive tract and embryos at the postimplantation egg cylinder stage (5½ and 6½ days *p.c.*) were removed from decidual swellings under a dissecting microscope in phosphate-buffered saline (PBS) or M2 medium (Quinn, Barros & Whittingham, 1982). The ectoplacental cone was removed, by gentle pipetting, from 6½-day embryos that were used to quantify the relative contribution of oocyte-coded enzyme, in order to avoid inclusion of any adhering maternal cells. However, since the ectoplacental cone is an embryonic-derivative, total GPI-1 activity was estimated for intact 6½-day embryos as well as for those without an ectoplacental cone. Unfertilized eggs were collected from the oviducts of unmated female mice that had been induced to ovulate. Cumulus cells were removed in a solution containing 100 units of hyaluronidase per ml of PBS or 0.9 % saline. Samples of eggs or embryos were stored at –20°C as described by West & Green (1983).

(C) *Electrophoresis and densitometry*

Cellulose acetate electrophoresis was done on 76×60 mm Helena, Titan III electrophoresis plates, using the pH 8.5 Tris-glycine buffer (3.0 g Tris base plus 14.4 g glycine per litre) described by Eicher & Washburn (1978). After electrophoresis for 1 h at 200 V the plates were stained and the proportions of the different GPI-1 allozymes were quantified by scanning densitometry as previously described (West & Green, 1983; West & Fisher, 1984). The technical controls reported by West & Green (1983) showed that this method was quantitatively reproducible and quite accurate.

(D) *Calculations*

The relative proportions of oocyte-coded and both maternally derived and paternally derived, embryo-coded GPI-1 were calculated for *Gpi-1s<sup>a</sup>/Gpi-1s<sup>b</sup>* embryos from the relative proportions of the three bands of GPI-1 activity that represent the GPI-1AA, GPI-1AB and GPI-1BB dimers (GPI-1A, GPI-1AB and GPI-1B allozymes) respectively. If the GPI-1A and GPI-1B monomers are co-ordinately synthesized and associate randomly to form dimers, the embryo-coded enzyme is expected to occur in the ratio  $p^2$  AA:2pq AB:q<sup>2</sup> BB, where p and q are the proportions of available embryo-coded A and B monomers and p+q = 1. The oocyte-coded enzyme (m) is exclusively GPI-1AA homodimer (GPI-1A allozyme), so when both oocyte and embryo-coded enzyme is present the allozymes occur in the ratio (p<sup>2</sup> + m) A:2pq AB:q<sup>2</sup> B and the sum of these is now greater than one.

The values of q and m were calculated from equations 4 and 5. The functions x<sub>1</sub>, x<sub>2</sub> and x<sub>3</sub> represent the observed proportions of the AA, AB and BB dimers (GPI-1A, GPI-1AB and GPI-1B allozymes), respectively, and are normalized so that x<sub>1</sub> + x<sub>2</sub> + x<sub>3</sub> = 1.

$$x_1 : x_2 : x_3 = p^2 + m : 2pq : q^2$$

$$p + q = 1$$

$$q^2 = kx_3 \quad (1)$$

$$2q(1 - q) = kx_2 \quad (2)$$

$$(1 - q)^2 + m = kx_1 \quad (3)$$

where k is a constant of proportionality.

From equations 1 and 2 by division:

$$\frac{q}{2(1 - q)} = \frac{x_3}{x_2}$$

$$qx_2 = 2(1 - q)x_3$$

$$= 2x_3 - 2qx_3$$

$$qx_2 + 2qx_3 = 2x_3$$

$$q(x_2 + 2x_3) = 2x_3$$

$$\therefore q = \frac{2x_3}{x_2 + 2x_3} \quad (4)$$

From equation 1,  $k = q^2/x_3$ . Substituting for k in equation 3:

$$m = kx_1 - (1 - q)^2$$

$$m = \frac{q^2x_1}{x_3} - (1 - q)^2 \quad (5)$$

## RESULTS

(A) *Genetic controls*

Two observations show that the 129 strain carries the same *Gpi-1t* temporal allele as the C57BL (B) strain. First, the mean proportions of allozymes produced

by 15 individual eggs from  $(129 \times B)F_1$  females was 23% GPI-1A:48% GPI-1AB:29% GPI-1B. Second, 14 samples each containing one 129-strain and one B-strain unfertilized egg produced allozymes in the ratio  $46.2 \pm 1.9\%$  A (129 strain):  $53.8 \pm 1.9\%$  B (B-strain). The *Gpi-It* genotype affects the GPI-1 activity in oocytes and unfertilized eggs so the following results for  $(129 \times B)F_1$  embryos are relevant only to *Gpi-1s<sup>a</sup>t<sup>b</sup>*/*Gpi-1s<sup>b</sup>t<sup>b</sup>* embryos.

### (B) *The transition from oocyte-coded to embryo-coded GPI-1*

The binomial analysis of the relative contributions of the oocyte-coded and the two embryonic *Gpi-1s* alleles makes the following assumptions: (a) Monomers are coordinately synthesized and associate at random to form enzymatically active dimers. (For example, if GPI-1A and GPI-1B monomers were synthesized at completely different times of the cell cycle in a *Gpi-1s<sup>a</sup>*/*Gpi-1s<sup>b</sup>* heterozygote, no GPI-1AB heterodimer would be expected.) (b) Constituent monomers are not reutilized after dimers are degraded.

#### (1) *Activation of the embryo-coded Gpi-1s genes*

Two patterns of GPI-1 allozymes were found in early  $(129 \times B)F_1$  mouse embryos (Table 1). Most of the embryos from 1-cell to approximately 8-cell stages (0.4 to 2.4 days) produced only the GPI-1A allozyme while the majority of later stage embryos produced the three-banded pattern expected once both of the embryo-coded *Gpi-1s* alleles are expressed (Fig. 1). No embryos produced GPI-1A and GPI-1B without GPI-1AB.

Five samples of 2.4-day embryos produced all three GPI-1 allozymes (Table 1). In each case the sample contained some embryos that had more than 8 cells. The electrophoresis plates were deliberately overstained in order to visualize any weakly staining allozymes. Consequently, sub-banding of the major GPI-1A allozyme occurred towards the origin (anode). The GPI-1AB and GPI-1B bands produced by the five samples were too weak to be quantified accurately but in three samples (17, 20 and 55 embryos respectively) the GPI-1B band appeared to be slightly stronger than GPI-1AB. In one large sample of 71 embryos GPI-1AB and GPI-1B were stained approximately equally and in the remaining sample of 10 embryos both bands were very faint. No GPI-1AB or GPI-1B allozymes were seen in two control samples comprising 48 and 53 unfertilized 129-strain eggs, collected 0.4 days after HCG injection.

The presence of both GPI-1AB and GPI-1B allozymes indicates that both of the embryo-coded *Gpi-1s* alleles were expressed in some of these 2.4-day embryos (assuming that GPI-1AB heterodimers are not synthesized from the breakdown of oocyte-coded GPI-1AA homodimers). The observation that in three cases, the GPI-1AB band stained less strongly than GPI-1B raises the possibility that the paternally derived *Gpi-1s<sup>b</sup>* allele may sometimes be activated before the maternally derived *Gpi-1s<sup>a</sup>* allele.

By 3.4 and 4.4 days *p.c.*, all three GPI-1 allozymes are produced by the majority of samples (Fig. 1). The occurrence of a few samples (mostly individual embryos)

that produced only GPI-1A might reflect weak staining, heterogeneity for embryonic stage or genuine heterogeneity for the time of onset of *Gpi-1s<sup>b</sup>* expression. Although obviously retarded embryos were excluded from the analysis, 3.4-day embryos, for example, included morulae and blastocysts. However, all 3.4-day embryos were further advanced than the most precocious 2.4-day embryos.

The quantitative analysis of GPI-1 allozyme patterns in 3.4- to 6.4-day embryos provides a means of estimating the relative contributions of (1) oocyte-coded GPI-1, (2) embryo-coded GPI-1 from the maternally derived *Gpi-1s<sup>a</sup>* allele and (3) embryo-coded GPI-1 from the paternally derived *Gpi-1s<sup>b</sup>* allele. The calculations assume that GPI-1AB and GPI-1B are binomially distributed as explained in the Materials and Methods section.

The results of the quantitative analysis are shown in Table 2. The relative contribution of the two embryo-coded *Gpi-1s* alleles (shown as p:q in Table 2) is similar in all samples.

Moreover the contribution of the maternally derived *Gpi-1s<sup>a</sup>* allele (p) was not significantly lower when the overall proportion of embryo-coded enzyme,  $(p+q)/(p+q+m)$ , was low (Fig. 2). Thus, there is no evidence from these samples that the paternally derived *Gpi-1s<sup>b</sup>* allele is usually activated earlier than the maternally derived *Gpi-1s<sup>a</sup>* allele.

Table 1. Frequency of multiple-banded GPI-1 allozyme patterns produced by  $(129\text{♀} \times B\text{♂})F_1$  embryos

Age (days p.c.)	Stage	Embryos per sample	No. of samples showing pattern	
			Only A band	A+AB+B bands
0.4	1-cell	20	1	0
1.4	2-cell	20, 30	2	0
2.4	4 to 8-cell	3 to 20†	6	1
2.4*	4 to 16-cell	10 to 71‡	1	4
2.6	Mostly 8-cell	19	1	0
3.4	Morula/ blastocyst	1	4	4
3.4	Morula/ blastocyst	3	2	14
3.6	Blastocyst	1	0	4
3.6	Blastocyst	3	0	14
4.4	Late blastocyst	1	2	16
4.4	Late blastocyst	3	0	9
5.4	Egg cylinder	1	0	7
6.4	Egg cylinder	1	0	14

\* These 2.4-day embryos were produced by induced ovulation. All of the others were the result of natural matings.

† Only the A band was detected in 3 groups of 3, 1 group of 10 and 2 groups of 20 embryos. All three allozyme bands were seen in another group of 20 embryos.

‡ 1 group of 11 embryos produced only the A band; all three bands were seen in another 4 groups (10, 17, 55 and 71 embryos respectively).

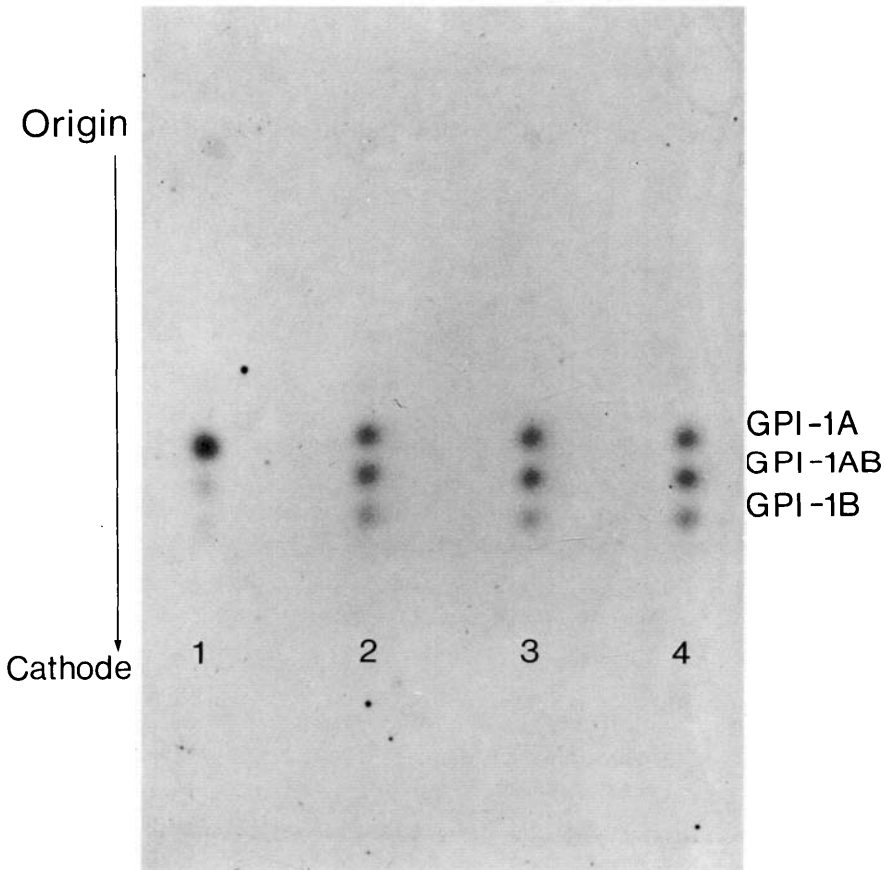


Fig. 1. GPI-1 allozymes produced by three 3.6-day embryos (track 1) and three samples of three 4.4-day embryos (tracks 2-4). The observed proportions of the GPI-1 allozymes was: track 1, 0.78 A:0.16 AB:0.06 B; track 2, 0.41 A:0.38 AB:0.21 B; track 3, 0.40 A:0.38 AB:0.21 B; track 4, 0.36 A:0.40 AB:0.23 B.

### (2) Decline of the oocyte-coded GPI-1 enzyme

The proportion of residual oocyte-coded enzyme can be estimated from the quantitative analysis (Table 2). The proportions of the three GPI-1 allozymes is close to 1:2:1 in 5.4- and 6.4-day embryos, indicating that little, if any, oocyte-coded GPI-1AA homodimer (GPI-1A allozyme) remains. The calculated proportions of oocyte-coded GPI-1 for 5.4- and 6.4-day embryos are 0.09 and 0.04 respectively. However, a similar calculation for unfertilized eggs from (129×B)<sub>F</sub><sub>1</sub> females also indicated a 'surplus' of 3% GPI-1A allozyme (Table 2). This gives some measure of the limitations of the calculations since all three GPI-1 allozymes in unfertilized eggs would be expected to be distributed binomially unless they have different stabilities. We, therefore, conclude that, within the limits of experimental error, the oocyte-coded GPI-1A allozyme is exhausted by 6.4 days *p.c.* There is likely to be some remaining at 5.4 days but this is probably less than the 9% shown in Table 2.

Table 2. Quantitative analysis of GPI-1 allozymes produced by  $(129\text{♀} \times B\text{♂})F_1$  embryos and unfertilized eggs from  $(129 \times B)F_1$  females. (Only samples producing all three GPI-1 allozymes are included.)

Age (days p.c.)	No. of embryos in sample	Observed proportions of allozymes (mean $\pm$ s.e.)			Calculated proportions*				Mean ratio of maternal: paternal embryo-coded GPI-1 p:q
		A ( $x_1$ )	AB ( $x_2$ )	B ( $x_3$ )	Oocyte-coded m/(m+p+q)	Embryo-coded maternal p/(m+p+q)	Embryo-coded paternal q/(m+p+q)		
<i>(129♀ × B♂)F<sub>1</sub> embryos</i>									
3.4	3	0.83 $\pm$ 0.02	0.11 $\pm$ 0.01	0.06 $\pm$ 0.01	0.79 $\pm$ 0.02 (range: 0.72-0.87)†	0.10 $\pm$ 0.01	0.11 $\pm$ 0.01		0.47:0.53
3.6	3	0.79 $\pm$ 0.02	0.14 $\pm$ 0.01	0.07 $\pm$ 0.01	0.72 $\pm$ 0.03 (0.59-0.83)	0.14 $\pm$ 0.01	0.14 $\pm$ 0.01		0.50:0.50
4.4	1	0.55 $\pm$ 0.06	0.29 $\pm$ 0.04	0.16 $\pm$ 0.02	0.42 $\pm$ 0.08 (0.15-0.77)†	0.27 $\pm$ 0.04	0.31 $\pm$ 0.04		0.47:0.53
4.4	3	0.39 $\pm$ 0.02	0.39 $\pm$ 0.01	0.21 $\pm$ 0.01	0.21 $\pm$ 0.01 (0.13-0.25)	0.38 $\pm$ 0.01	0.41 $\pm$ 0.01		0.48:0.52
5.4	1	0.27 $\pm$ 0.01	0.45 $\pm$ 0.01	0.28 $\pm$ 0.01	0.09 $\pm$ 0.02 (0.02-0.14)	0.41 $\pm$ 0.01	0.50 $\pm$ 0.01		0.45:0.55
6.4	1	0.23 $\pm$ 0.01	0.47 $\pm$ 0.01	0.30 $\pm$ 0.01	0.04 $\pm$ 0.02 (-0.05-+0.12)	0.43 $\pm$ 0.02	0.53 $\pm$ 0.003		0.44:0.56
<i>Unfertilized eggs from (129 × B)F<sub>1</sub> ♀♀. (Control for limits of calculation.)</i>									
	1	0.23 $\pm$ 0.01	0.48 $\pm$ 0.004	0.29 $\pm$ 0.01					
	15								0.03 $\pm$ 0.01‡ (-0.01-+0.07)

\* See Materials and Methods for details of calculations.

† Samples producing only oocyte-coded GPI-1 were not included in the quantitative analysis. See Table 1.

‡ Theoretical value is zero if there is no 'surplus' GPI-1A allozyme.

Table 2 indicates that at 3.4 days 79 % of the GPI-1 activity is oocyte coded. This figure is increased to 82 % by correcting for the occurrence of 2/16 samples of three 3.4-day embryos that produced only oocyte-coded enzyme (Table 1). The estimated proportion of oocyte-coded enzyme is much more variable for single 4.4-day embryos than for groups of three (Table 2). This heterogeneity among individual embryos at 4.4 days probably reflects rapid increases in the proportion of newly synthesized embryo-coded GPI-1 in embryos with little remaining oocyte-coded activity.

The kinetics of the decline in oocyte-coded GPI-1 enzyme was investigated by converting the proportion of oocyte-coded enzyme to the activity of oocyte-coded enzyme. The activity of GPI-1 in embryos was compared to the activity produced by known numbers of unfertilized 129-strain eggs. The embryos and eggs were applied to adjacent tracks on an electrophoresis plate and the electrophoresis time was reduced to 30 min at 100 V in order to prevent separation of the three GPI-1 allozymes in the embryos. The relative proportions were then estimated by scanning across the tracks as previously described (West & Fisher, 1984). Preliminary experiments involving comparisons of different numbers of eggs showed that quantitation was satisfactory provided that the activities in the two samples was not too unequal. For this reason single unfertilized eggs were used to estimate relative activities in 0.4- to 4.4-day embryos but groups of 6 and 20 eggs were used for 5.4- and 6.4-day embryos respectively.

The relative GPI-1 activities (Table 3) were converted to total GPI-1 activities per embryo (Table 4) as described in the footnote to Table 4. These values and the

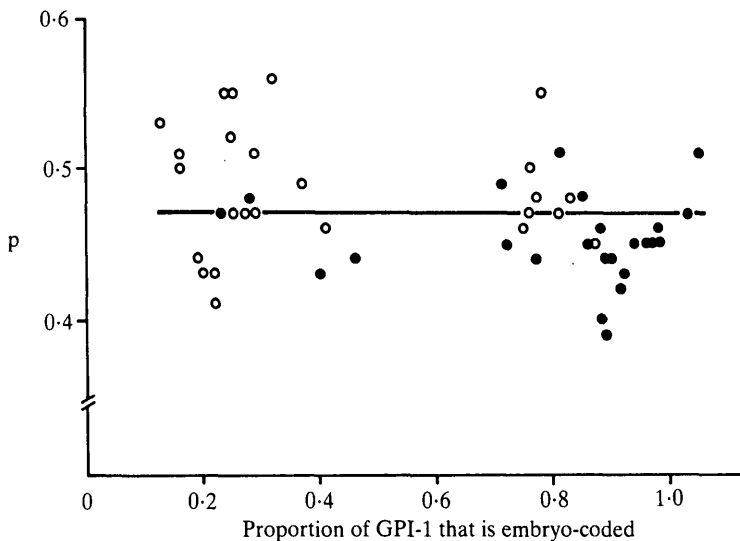


Fig. 2. The proportion ( $p$ ) of embryo-coded GPI-1 that is encoded by the maternally derived *Gpi-1s<sup>a</sup>* allele plotted against the proportion,  $(p+q)/(p+q+m)$ , of GPI-1 that is embryo coded, for individual samples of 3.4- to 6.4-day embryos. Groups of three embryos are shown as open circles and single embryos are represented by closed circles. The horizontal line indicates the overall mean value of  $p = 0.47$ .



estimates of the proportion of GPI-1 that was oocyte-coded were used to derive estimates of the oocyte-coded GPI-1 activity. These data (Table 4 and Fig. 3) show that the oocyte-coded GPI-1 activity is fairly constant up to 2.4 days but declines thereafter. The decline is most rapid between 3.4 and 4.4 days *p.c.* when the half life of the oocyte-coded enzyme appears to be close to 12h. The total GPI-1 activity falls to a minimum around 3.4 to 4.4 days *p.c.* even though embryo-coded expression has begun.

Table 3. *Estimated GPI-1 activity in (129×B)F<sub>1</sub> embryos relative to activity in a single unfertilized 129-strain egg*

Age of embryo	No. of samples	Relative GPI-1 activity* (mean ± s.e.)
0.4	8	0.90 ± 0.04
1.4	10	1.14 ± 0.07
2.4	7	0.99 ± 0.05
3.4	8	0.66 ± 0.04
4.4	8	0.63 ± 0.05
5.4	7	6.22 ± 0.66
6.4 (without ectoplacental cone)	6	34.33 ± 2.68
6.4 (with ectoplacental cone)	6	48.97 ± 2.96

\* The activity in single embryos was compared to the activity in 6 eggs for 5.4-day embryos, 20 eggs for 6.4-day embryos and single eggs for all other embryos.

Table 4. *Estimates of oocyte-coded and embryo-coded GPI-1 activity in (129×B)F<sub>1</sub> embryos*

Age (days <i>p.c.</i> )	% Oocyte-coded GPI-1 activity*	Estimated GPI-1 activity (nmol embryo <sup>-1</sup> h <sup>-1</sup> )		
		Total†	Oocyte-coded	Embryo-coded
0.4	100	1.17	1.17	0
1.4	100	1.48	1.48	0
2.4	≤100	1.29	≤1.29	≥0
3.4	82	0.86	0.71	0.15
4.4	21	0.82	0.17	0.65
5.4	≥0	8.09	≥0	≤8.09
6.4	0	63.66	0	63.66

\* Although embryo-coded GPI-1 was detected in some 2.4-day embryos this represented a negligible proportion overall. Data for 3.4- and 4.4-day embryos are from Table 2 (groups of 3) but the % oocyte-coded GPI-1 in 3.4-day embryos was corrected to allow for 2/16 samples that produced no embryo-coded enzyme. Values given in Table 2 for 5.4- and 6.4-day embryos were too close to the limits of the calculation to be reliable.

† The total GPI-1 activity was estimated from the relative activity (Table 3) assuming that the mean activity for a 129 strain unfertilized egg is 1.3 nmol NADPH h<sup>-1</sup>. (This is calculated from published figures for C57BL and C57BL/6 eggs (Peterson & Wong, 1978; McLaren & Büehr, 1981) corrected for our observation that 129 eggs have 86 % of the activity of C57BL eggs.) The activities for 6.4-day embryos include the ectoplacental cone.

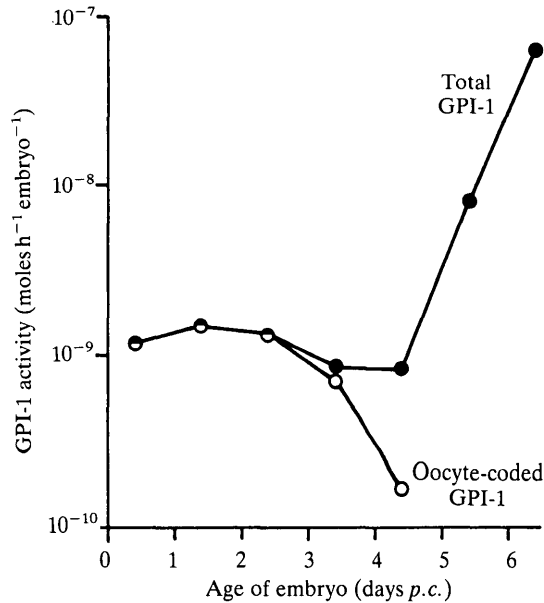


Fig. 3. Change in total and oocyte-coded GPI-1 activity during development of (129×B)<sub>F</sub><sub>1</sub> embryos.

#### DISCUSSION

The quantitative analysis of GPI-1 allozymes in early mouse embryos described here has enabled us to dissect the relative contributions of the oocyte-coded and the two embryo-coded *Gpi-1s* alleles. This approach complements other methods that require either (1) the existence of more than two alleles that code for electrophoretic variants (West & Green, 1983; Duboule & Bürki, 1985) or (2) sophisticated nuclear transfer experiments (Gilbert & Solter, 1985). As such, the method should prove generally applicable for the analysis of expression of other dimeric enzymes with known allozyme variants.

The relative timing of activation of the two embryo-coded alleles has previously been investigated for the X-linked gene *Pgk-1* as well as for autosomal *Gpi-1s* in the mouse. The maternally derived *Pgk-1* allele that encodes the enzyme phosphoglycerate kinase appears to be expressed before the paternally derived allele (Papaioannou *et al.* 1981; Krietsch *et al.* 1982). In contrast, various lines of evidence suggest that, for *Gpi-1s*, the two embryo-coded alleles are usually activated synchronously.

Two new allozymes (representing new homodimer and heterodimer) are detected as soon as embryo-coded enzyme appears. This was reported for 2½-day embryos (Brinster, 1973, and our present results) and later stage embryos (Chapman *et al.* 1971; West & Green, 1983; Doboule & Bürki, 1985; Gilbert & Solter, 1985). Quantification of the allozyme bands produced by 3·4-day (and older) embryos showed no tendency for the paternally derived *Gpi-1s* allele to contribute more GPI-1 enzyme than the maternally derived allele in embryos with

relatively little embryo-coded enzyme (Fig. 2). Both of these observations suggest that the paternally derived allele is not usually activated before the maternal allele but, because of the presence of oocyte-coded enzyme, they do not tell us whether the maternally derived allele is activated first.

Experiments with three *Gpi-1s* alleles (West & Green, 1983; Duboule & Bürki, 1985) and experiments involving transplantation of pronuclei (Gilbert & Solter, 1985) distinguish between oocyte-coded GPI-1 enzyme and that encoded by the maternally derived embryonic gene. These experiments indicate that the maternally derived, embryo-coded *Gpi-1s* allele is not normally activated before the paternally derived allele.

Together, these experiments imply that the two embryo-coded *Gpi-1s* alleles are usually activated synchronously. However, our observation of three groups of 2.4-day embryos with a weak GPI-1B and still weaker GPI-1AB band suggests, but does not prove, that the paternally derived *Gpi-1s<sup>b</sup>* allele is sometimes activated before the maternally derived *Gpi-1s<sup>a</sup>* allele in the particular cross studied. It, therefore, remains possible that the embryonic *Gpi-1s* genes are activated independently but usually simultaneously so that one or other allele may sometimes, by chance, be activated fractionally earlier.

Embryo-coded gene product is consistently found at 3½ days *p.c.* (West & Green, 1983; Duboule & Bürki, 1985; Gilbert & Solter, 1985) and has been detected in *Gpi-1s<sup>a</sup>/Gpi-1s<sup>b</sup>* embryos at 2½ days *p.c.* on two occasions (Brinster, 1973; Table 1 of this report). Embryonic expression of the *Gpi-1s* gene must occur before the enzyme is first detected and so, in some genotypes at least, it must occur at or before 2½ days *p.c.* In our experiments some embryos had advanced beyond the 8-cell stage by 2.4 days so we cannot be certain that embryo-coded GPI-1 is detectable in 8-cell embryos.

Analysis of the oocyte-coded enzyme showed that there was not a uniform rate of loss of activity throughout early development. Rather, the activity remained roughly constant until 2½ days *p.c.* and then declined sharply. Our present experiments support previous evidence (West & Green, 1983; Duboule & Bürki, 1985; Gilbert & Solter, 1985) that the oocyte-coded enzyme is exhausted by 5½ to 6½ days *p.c.* Embryo-coded GPI-1 begins to make a significant contribution to the total activity almost immediately the oocyte-coded enzyme declines. The total GPI-1 activity falls to a minimum around 3.4 to 4.4 days and then rises steeply. The rapid rise in GPI-1 activity per embryo is similar to that seen for phosphoglycerate kinase (Papaioannou *et al.* 1981) and coincides with a period of rapid cellular proliferation (McLaren, 1976; Snow, 1976).

The developmental profile of GPI-1 activity, shown in Fig. 3, is similar to the patterns documented by Harper & Monk (1983) for total activities of the enzymes lactate dehydrogenase, phosphoglycerate kinase and glucose-6-phosphate dehydrogenase in mouse embryos. These authors proposed that the decline in activity of these enzymes around 2½ days of development represents some common mechanism for removing oocyte-coded gene products which directs the transition from oocyte-coded to embryo-coded enzymes. GPI-1 shows a similar pattern and

it is tempting to speculate that the constant GPI-1 activity during the first  $2\frac{1}{2}$  days of development is maintained by translation of pre-existing oocyte-coded mRNA present in the embryo before  $2\frac{1}{2}$  days *p.c.* Analysis of polypeptide synthesis in early mouse embryos (Flach *et al.* 1982; Bolton, Oades & Johnson, 1984) suggests that some oocyte-coded mRNAs are stable for the first 25 h but may be inactivated in the late 2-cell embryo ( $1\frac{1}{2}$  to 2 days *p.c.*). A similar decline in oocyte-coded GPI-1 mRNA at this stage would be followed by a decline in oocyte-coded GPI-1 enzyme, as observed from 2-4 days.

Electrophoresis experiments (West & Green, 1983; Duboule & Bürki, 1985; Gilbert & Solter, 1985) suggest that no oocyte-coded GPI-1 mRNA is present when the embryo-coded *Gpi-1s* genes are first expressed. It is not known whether such mRNA is present in younger embryos but this problem could be approached by DNA-RNA or RNA-RNA *in situ* hybridization.

#### CONCLUSIONS

(1) Oocyte-coded GPI-1 is stable until  $2\frac{1}{2}$  days *p.c.* Thereafter it declines in activity and is exhausted by  $5\frac{1}{2}$  to  $6\frac{1}{2}$  days *p.c.*

(2) Embryo-coded GPI-1 is readily detectable at  $3\frac{1}{2}$  days and was detected in several samples of  $2\frac{1}{2}$ -day embryos.

(3) The maternally and paternally derived, embryo-coded *Gpi-1s* genes are probably usually activated synchronously but small differences in timing may occur and the two alleles could be activated independently.

(4) Total GPI-1 activity falls to a minimum around  $3\frac{1}{2}$  to  $4\frac{1}{2}$  days *p.c.* even though embryo-coded gene expression has begun.

(5) The profile of oocyte-coded GPI-1 activity during early mouse development is consistent with the suggestion, made by Harper & Monk (1983), that there is a common mechanism for the removal of oocyte-coded gene products at around  $2\frac{1}{2}$  days *p.c.*

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