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Genetic differences in glucose phosphate isomerase activity among mouse embryos

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Summary

We have compared mouse embryos of three heterozygous, congenic genotypes (with high, medium and low levels of oocyte-coded glucose phosphate isomerase (GPI-1) activity respectively) to test whether 1) the survival time of oocyte-coded GPI-1 activity in the early embryo is affected by its activity level in the oocyte and 2) whether embryo-coded GPI-1 is detected earlier in embryos that inherit low levels of oocyte-coded GPI-1. The oocyte-coded GPI-1 was entirely GPI-1A allozyme in the high and medium groups but was the less stable GPI-1C allozyme in the low group. We determined total GPI-1 activity and the ratio of different GPI-1 allozymes in early embryos and calculated the activity of oocyte-coded and embryo-coded GPI-1. In all three groups, the oocyte-coded enzyme activity remained at a more or less constant level for the first 21½ days. Some oocyte-coded GPI-1 remained in 4½ day embryos from the high and medium groups but was gone by 5½ days. Very little remained in 4½ day embryos that inherited low levels of a less stable form of the enzyme (GPI-1C allozyme). Despite a 4- to 5-fold difference in initial oocyte-coded GPI-1 activity, no differences were seen among the three genotypically distinct groups of embryos in the time of activation of the embryonic *Gpi-1s* genes. The embryo-coded GPI-1 was first detectable in 3½ day compacted morulae in all three groups. The level of oocyte-coded GPI-1, in the high group, when embryo-coded GPI-1 was first detected was higher than the level in the low group at any stage prior to detection of embryo-coded GPI-1. These results imply that embryonic *Gpi-1s* genes are not activated by a mechanism that depends on oocyte-coded GPI-1 enzyme activity falling below a critical threshold. Once both embryonic *Gpi-1s* alleles were activated, they were usually equally expressed in $Gpi\text{-}1s^a/Gpi\text{-}1s^b$ heterozygous embryos. However, the maternal: paternal expression was closer to 2:1 in 5% (3/60) of the 5½ and 6½ day embryos. These could have been digynic triploid embryos.

Key words: mouse, embryo, glucose phosphate isomerase, gene activation, genetic variants, electrophoresis.

Introduction

Embryonic genes are activated during the early stages of mammalian development and the oocyte-coded gene products are replaced by embryo-coded products. The transition from oocyte-coded to embryo-coded glucose phosphate isomerase (GPI-1; E.C. 5.3.1.9) has been extensively studied in mouse embryos (Chapman *et al.* 1971; Brinster, 1973; West and Green, 1983; Duboule and Burki, 1985; Gilbert and Solter, 1985; West *et al.* 1986). These studies have shown that embryo-coded GPI-1 is usually first detected at 3½ days (or occasionally 2½ days) *post coitum (p.c.)* and that maternally and paternally derived *Gpi-1s* alleles are probably activated synchronously. (The symbol '*Gpi-1s*' refers to the structural gene encoding GPI-1 enzyme and has replaced the former symbol '*Gpi-1*'.) Oocyte-coded GPI-1 activity is relatively stable until 2½ days and then declines and is exhausted by 5½ to 6½ days *p.c.* The total GPI-1 activity falls to a minimum in the 3½ to 4½ day blastocyst even though embryo-coded expression has begun.

These studies have provided a quantitative description of the transition from oocyte-coded to embryo-coded GPI-1 and also imply that oocyte-coded GPI-1 mRNA is not present when the embryonic *Gpi-1s* genes are expressed (West and Green, 1983; Duboule and Burki, 1985; Gilbert and Solter, 1985). However, the mechanisms involved in activating the embryonic *Gpi-1s* genes remain unknown. This may involve interaction of genetic elements (e.g. promoter or enhancer sequences) with cellular factors that provide developmental cues (for discussion see Rosenfeld *et al.* 1987). For example, the activation of the gene encoding the LDH-5 (A4) isozyme of lactate dehydrogenase seems to be triggered by the proliferation of trophoblast after implantation (Auerbach and Brinster, 1967; Monk and Petzoldt, 1977; Speilman *et al.* 1978).

Although embryo-coded GPI-1 activity has not usually been detected before the morula stage, Brinster

(1973) detected it in 8-cell-stage embryos. This discrepancy could reflect genetic differences between the strains of mice used in different studies. Genetic differences could affect either the promoter/enhancer sequences or the developmental cues and so alter the time of gene activation. Autoregulation of gene expression occurs in some eukaryotic systems (Serfling, 1989) and it is possible that the falling level of oocyte-coded GPI-1 acts as a developmental cue that triggers expression of the embryonic *Gpi-1s* genes in the preimplantation embryo. The oocytes of some strains of mice have low levels of GPI-1 (Peterson and Wong, 1978) and it is possible that this could trigger the early expression of embryonic *Gpi-1s* genes.

In the present study, we have compared the transition from oocyte-coded to embryo-coded GPI-1 in mouse embryos that inherited high, medium or low levels of oocyte-coded GPI-1 activity. These comparisons were made in order to address two questions. First, we asked whether the survival time of oocyte-coded GPI-1 activity, in the early embryo, is affected by the activity level in the oocyte. Second, we asked whether embryo-coded GPI-1 is detected earlier in embryos that inherit low levels of oocyte-coded GPI-1.

Materials and methods

Mice

The following strains of mice (with abbreviated strain designations in parentheses) were maintained under conventional conditions in the Centre for Reproductive Biology: inbred strain C57BL/Ola (B); partially congenic strains (8 backcross generations) C57BL/Ola.129-*Gpi-1s*^{a}/Ws (B-*Gpi-1s*^{a}), C57BL/Ola.AKR-c *Gpi-1s*^{a}/Ws (B-*c*), C57BL/Ola-*Gpi-1s*^{c}/Ws (B-*Gpi-1s*^{c}) and C57BL/Ola-*Gpi-1s*$^{a\text{-}m1H}$/Ws (B-null). The B-null stock carries the *Gpi-1s*$^{a\text{-}m1H}$ null allele (Peters and Ball, 1986) and was produced by crossing a *Gpi-1s*^{a}/*Gpi-1s*$^{a\text{-}m1H}$ (a/null) heterozygote (gift of Dr J.Peters) with C57BL/Ola (B). Heterozygous *Gpi-1s*^{b}/*Gpi-1s*$^{a\text{-}m1H}$ (b/null) offspring were crossed with B-*Gpi-1s*^{a} and subsequent generations crossed alternately to B and B-*Gpi-1s*^{a} strains (14 generations). The other stocks are described more fully by West and Fisher (1984).

Egg and embryo collection

Ovulation was induced by intraperitoneal injections of 5 i.u. pregnant mares' serum gonadotrophin (PMS) at approximately 12 noon followed 48 h later by 5 i.u. human chorionic gonadotrophin (hCG). Unfertilized eggs were collected from the oviducts of females approximately 21–23 h after hCG injection and handled and stored as previously described (West and Fisher, 1984; West *et al.* 1986). Unfertilized eggs were collected from females of strains B, B-*Gpi-1s*^{a}, B-*c*, B-*Gpi-1s*^{c} and DBA, from various heterozygotes produced by intercrossing these strains and from *Gpi-1s*^{a}/*Gpi-1s*$^{a\text{-}m1H}$ (*a*/null) heterozygotes. Samples comprising 4 (B-*Gpi-1s*^{c}), 2 (DBA) or individual (all other genotypes) unfertilized eggs were stored for GPI-1 densitometric assays.

Embryos were produced by mating superovulated B-*c*, B-*Gpi-1s*^{a}, or B-*Gpi-1s*^{c} strain females with B males on the day of the hCG injection. Embryos were collected between 9am and 12 noon, so that those collected on the day after the hCG injection (*i.e.* the day of the vaginal plug) were designated ½ day *p.c.* Preimplantation embryos (½ to 4½ days) were flushed from the reproductive tract and postimplantation embryos (5½ and 6½ day egg cylinders) were dissected from the decidual swellings in M2 medium (Quinn *et al.* 1982) as previously described (West *et al.* 1986). Samples of eggs or embryos were stored at −20°C as described by West and Green (1983). All samples were frozen and thawed three times in liquid nitrogen vapour before electrophoresis.

Postimplantation embryos for electrophoretic quantification of allozymes were stored individually but samples of ½ to 4½ day embryos usually comprised 3 (B-*c*×B)F_1 embryos, 3 (B-*Gpi-1s*^{a}×B)F_1 embryos or 4 to 5 (B-*Gpi-1s*^{c}×B)F_1 embryos. Samples of embryos for GPI-1 activity assays were either individual (B-*c*×B)F_1 or (B-*Gpi-1s*^{a}×B)F_1 embryos (½ to 4½ days *p.c.*) or groups of (B-*Gpi-1s*^{c}×B)F_1 embryos (5 per group at 3½ days and 3 per group at ½ to 2½ and 4½ days *p.c.*).

Glucose phosphate isomerase electrophoresis

Samples of embryos were applied directly from fine Pasteur pipettes to 76×60 mm Helena, Titan III cellulose acetate electrophoresis plates. Electrophoresis, staining for GPI-1 activity and quantification of the different GPI-1 allozymes was done as previously described (West *et al.* 1986). When one allozyme is present as a very low proportion of the total GPI-1 the proportion may, for technical reasons, be overestimated (see below and West and Green, 1983).

Glucose phosphate isomerase densitometric assays

The activity of GPI-1 in samples of eggs or preimplantation embryos was estimated relative to that in standard samples that contained a known number of unfertilized C57BL/Ola strain eggs by scanning densitometry as described by West *et al.* (1989). The activity is expressed relative to the activity in one C57BL/Ola unfertilized egg. (Peterson *et al.* (1985) have shown that for C57BL/6J eggs this is 1.92 nmol NADPH/egg/hour at 35°C.) Control experiments (West *et al.* 1989) showed that quantification by this method was excellent when the activity of the test sample was between one and three times that in the standard sample (control results for groups of 1, 2 and 3 eggs were 1.07±0.05, 2.05±0.13 and 2.91±0.12, respectively). When the ratio of the two samples was 4:1 or 5:1 the minor component tended to be slightly overestimated (control results were 3.66±0.15 and 4.52±0.24, respectively). In practice, a single B strain egg was used as a standard for all test samples and the number of eggs or embryos in the test sample was adjusted either to approximately match the expected GPI-1 activity of a single B strain egg or fall within the linear range (between one third and three times the standard sample).

Results

GPI-1 activity in unfertilized eggs

Comparison of unfertilized eggs from females of various genotypes (Table 1) revealed a wide range in GPI-1 activity. Three strains, B-*c* (high activity), B-*Gpi-1s*^{a} (medium activity) and B-*Gpi-1s*^{c} (low activity) were selected for the comparative analysis of the transition from oocyte-coded to embryo-coded GPI-1.

Changes in GPI-1 activity in embryos

Table 2 shows the change in total GPI-1 activity during preimplantation development of the three genotypes chosen for study. In both (B-*Gpi-1s*^{a}×B)F_1 and (B-*Gpi-*

Table 1. *Relative glucose phosphate isomerase activity in unfertilized eggs from female mice of different genotypes*

Strain	*Gpi-1s*	*Gpi-1t**	*n*	Mean relative GPI-1 activity
Inbred and congenic females				
B-*c*	a/a	c/c	10	1.77±0.07
B	b/b	b/b	10	1.07±0.05
B-*Gpi-1s*^{a}	a/a	b/b	12	0.85±0.04
DBA	a/a	a/a	9	0.31±0.02
B-*Gpi-1s*^{c}	c/c	?	10	0.35±0.02
Heterozygous females				
B-*Gpi-1s*^{a}×B	a/b	b/b	14	0.91±0.05
B-*Gpi-1s*^{c}×B	c/b	?/b	16	1.03±0.05
B-*Gpi-1s*^{c}×B-*Gpi-1s*^{a}	c/a	?/b	16	0.63±0.03
B-*Gpi-1s*^{c}×B-null	a/0	b/0	13	0.41±0.01

*See West and Fisher (1984) for further details of *Gpi-1t* genotypes.

$1s^c$×B)F_1 embryos, GPI-1 levels reach a minimum at 3½ days (morula and blastocyst stages)and then rise again at 4½ days (hatched blastocyst). The GPI-1 activity in (B-*c*×B)F_1 embryos also declines at 3½ days. Although there is a 4-fold difference in GPI-1 activity between the three genotypes at ½ day, by 4½ days embryos in all 3 groups have similar total GPI-1 activities.

Changes in GPI-1 allozymes in embryos

Representative densitometric tracings of electrophoresis plates are shown in Fig. 1. Table 3 shows the changes in the proportions of GPI-1 allozymes during the first week of development of (B-*c*×B)F_1 embryos (high oocyte-coded GPI-1 activity). Only the oocyte-coded A allozyme (AA dimer) was detected at ½ to 2½ days and in about half of the samples at 3½ days *p.c.* One sample of two 3½ day blastocysts produced an additional weak AB band but no detectable B allozyme. The remaining 3½ to 6½ day samples all produced all three allozymes. All but one of the postimplantation samples (5½ and 6½ days) produced allozymes close to the 1:2:1 ratio expected if no oocyte-coded GPI-1 remained and GPI-1 was entirely embryo-coded. The exceptional 6½ day embryo produced allozymes closer to the ratio 4A:4AB:1B. The proportion of total GPI-1 activity that is oocyte-coded was calculated in two ways (Table 3). When no oocyte-coded GPI-1 remains the expected allozyme ratio is 1A:2AB:1B (as seen in most 5½ and 6½ day embryos), so the proportion of embryo-coded GPI-1 is approximately twice the proportion of the AB allozyme; the proportion of oocyte-coded GPI-1 is then 1 minus the embryo-coded proportion. The second calculation makes use of the proportions of all three allozymes to derive the proportion of oocyte-coded GPI-1 from the formula m/(m+p+q) as noted in Table 3 and explained by West *et al.* (1986). The two calculations produced similar estimates of the proportion of residual oocyte-coded GPI-1. The final column of Table 3 shows the estimated contributions of the maternally derived *Gpi-1s*^{a} allele and the paternally derived *Gpi-1s*^{b} allele to the embryo-coded GPI-1 activity (the calculation is explained by West *et al.* 1986). Apart from the one anomolous 6½ day embryo, mentioned above, the two alleles are expressed to a similar extent. However, in these experiments, it was not possible to test whether the maternally derived, embryo-coded *Gpi-1s* allele was activated before the paternally derived allele.

Table 4 shows the equivalent analysis for the (B-*Gpi-1s*^{a}×B)F_1 embryos (medium oocyte-coded GPI-1 activity). The overall pattern is similar to the (B-*c*×B)F_1 embryos (Table 3) but embryo-coded enzyme was detected in a higher proportion of 3½ day samples (8/10 compared to 11/21). Again, one 3½ day sample produced a strong A band, a weak AB band but no B allozyme, but all three allozyme bands were visible in the majority of samples. Most 5½ and 6½ day embryos produced allozymes close to the expected 1:2:1 ratio but, as for the (B-*c*×B)F_1 embryos, there were exceptions. Two 5½ day (B-*Gpi-1s*^{a}×B)F_1 embryos had allozyme ratios close to 4A:4AB:1B. Apart from the exceptions noted, the expression of the maternally derived, embryo-coded *Gpi-1s* allele was approxi-

Table 2. *Change in relative GPI-1 activity during preimplantation development of embryos from three crosses (female×male)*

Age (days *p.c.*)	Stage	Mean relative GPI-1 activity±S.E. (*n*)		
		B-*c*×B	B-*Gpi-1s*^{a}×B	B-*Gpi-1s*^{c}×B
0.5	1-cell	1.30±0.11 (15)	0.75±0.08 (7)	0.36±0.03 (12)
1.5	2-cell	1.43±0.09 (13)	0.92±0.15 (7)	0.33±0.02 (10)
2.5	6- to 12-cell	1.35±0.01 (11)	0.72±0.04 (14)	0.29±0.01 (10)
3.5	compacted morula	1.23±0.17 (12)	0.38 (1)	0.20±0.02 (4)
3.5	blastocyst	0.74±0.16 (4)	0.37±0.03 (11)	0.23±0.02 (7)
4.5	hatched blastocyst	0.70±0.05 (17)	0.69±0.13 (11)	0.62±0.04 (11)

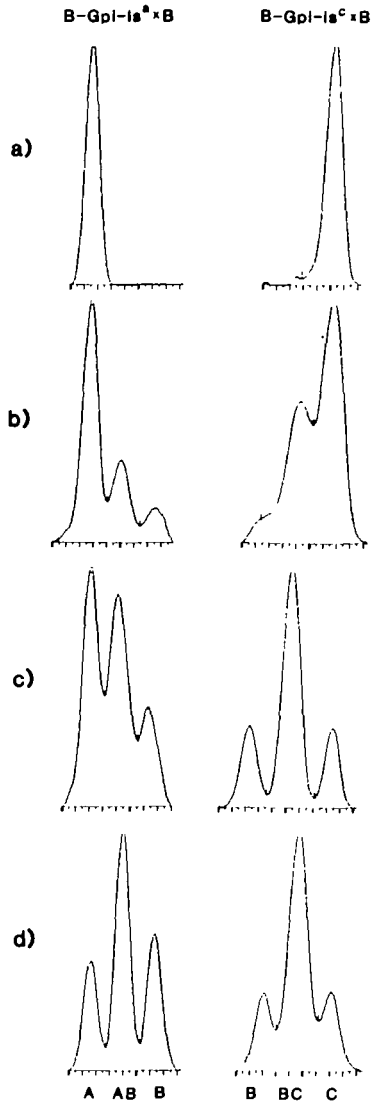

Fig. 1. Representative densitometric tracings of GPI-1 allozymes in (B-*Gpi-1s*^{a}×B)F_1 and (B-*Gpi-1s*^{c}×B)F_1 embryos showing the transition from oocyte-coded to embryo-coded enzyme. The peaks represent the proportions of the different GPI-1 allozymes (A, AB and B or B, BC, and C) in embryos at (a) 2½ days, (b) 3½ days, (c) 4½ days and (d) 5½ days *post coitum*.

mately equal to that for the paternally derived, embryo-coded allele (p:q ratios in Tables 3 and 4).

Table 5 shows the results for the (B-*Gpi-1s*^{c}×B)F_1 embryos (low oocyte-coded GPI-1 activity). Some ½ day samples and two 3½ day samples produced a strong C allozyme, a weak B allozyme but no CB allozyme. (It was not certain whether the weak band was a genuine B allozyme because it often seemed to be a little further from the C allozyme than is usual for a B allozyme.) This could be either a technical artefact or, for example, a result of transient expression of sperm GPI-1 mRNA in ½ day embryos or early expression of the paternally derived *Gpi-1s*^{b} allele in the 3½ day embryos. To test this, samples containing five unfertilized B-*Gpi-1s*^{c} eggs or five ½ day (B-*Gpi-1s*^{c}×B)F_1 embryos were run in alternate positions on the same electrophoresis plates. Some of both the unfertilized eggs (3/11 samples) and the embryos (4/11 samples) produced a weak extra band. It therefore seems probable that the extra band in seven of the eighteen ½ day embryos and in two of the fourteen 3½ day embryos, shown in Table 5, was a trailing sub-band of GPI-1C or another technical artefact rather than a genuine GPI-1B allozyme.

The other ½ to 2½ day (B-*Gpi-1s*^{c}×B)F_1 samples only produced the oocyte-coded C allozyme and the other 3½ day samples (12/14) produced all three allozyme bands. Similarly all 4½ and 5½ and all but one of the 6½ day embryos produced all 3 allozymes. (The anomalous 6½ day embryo had no detectable C allozyme and this probably reflects the greater lability of this allozyme.)

Table 3. *Change in GPI-1 allozyme ratios during preimplantation development of (B-c×B)F_1 embryos (a/a females×b/b males)*

Age in days *p.c.* (& group*)	Stage	No. of samples (embryos)	Observed percentage of allozyme (Mean±S.E.) A ($100x_1$)	AB ($100x_2$)	B ($100x_3$)	Calculated proportion of oocyte-coded GPI-1** $1-2x_2$	$m/(m+p+q)$	Maternal:paternal embryo-coded GPI-1 p:q
0.5 (i)	1-cell	6 (18)	100±0	0±0	0±0	1.0	–	–
1.5 (i)	2-cell	7 (21)	100±0	0±0	0±0	1.0	–	–
2.5 (i)	6- to 12-cell	8 (37)+	100±0	0±0	0±0	1.0	–	–
3.5 (i)	compacted morula	1 (4)	100	0	0	1.0	–	–
3.5 (i)	blastocyst	9 (27)	100±0	0±0	0±0	1.0	–	–
3.5 (ii)	blastocyst	1 (2)	94.1	5.9	0	0.88	–	–
3.5 (iii)	compacted morula	1 (4)	92.0	5.3	2.6	0.89	0.89	50:50
3.5 (iii)	blastocyst	9 (27)	84.3±1.3	10.6±0.8	5.0±0.8	0.79±0.02	0.76±0.02	53:47
4.5 (iii)	hatched blastocyst	9 (26)	51.3±5.2	32.4±3.2	16.2±2.3	0.35±0.06	0.34±0.06	51:49
5.5 (iv)	egg cylinder	15 (15)	27.2±0.9	48.4±0.4	24.4±1.0	0.03±0.01	0.02±0.01	50:50
6.5 (iii)	egg cylinder	1 (1)	44.8	42.4	12.8	0.15	0.10	62:38
6.5 (iv)	egg cylinder	14 (14)	24.7±0.3	48.12±0.4	27.3±0.4	0.04±0.01	0.03±0.01	47:53

* Grouped according to the number of allozyme bands present. Groups iii and iv both have 3 allozymes but in group iv the ratios are close to 25:50:25 (little or no remaining oocyte-coded GPI-1).

** The proportion of oocyte-coded GPI-1 was calculated in two ways. The functions p, q and m were calculated as follows: $q=2x_3/(x_2+2x_3)$; $p=1-q$; $m=(q^2x_1/x_3)-(1-q)^2$. See West *et al* 1986 for derivation of formulae.

+ 7 groups of 3 embryos plus one group of 16 embryos.

Table 4. *Change in GPI-1 allozyme ratios during preimplantation development of (B-*Gpi-1s^a×B)F$_1$ embryos (a/a *females*×b/b *males)*

Age in days *p.c.* (& group*)	Stage	No. of samples (embryos)	Observed percentage of allozyme (Mean±S.E.) A ($100x_1$)	AB ($100x_2$)	B ($100x_3$)	Calculated proportion of oocyte-coded GPI-1** $1\text{-}2x_2$	$m/(m+p+q)$	Maternal:paternal embryo-coded GPI-1 p:q
0.5 (i)	1-cell	11 (33)	100±0	0±0	0±0	1.0	–	–
1.5 (i)	2-cell	11 (33)	100±0	0±0	0±0	1.0	–	–
2.5 (i)	6- to 12-cell	10 (30)	100±0	0±0	0±0	1.0	–	–
3.5 (i)	compacted morula	1 (4)	100	0	0	1.0	–	–
3.5 (i)	blastocyst	1 (5)	100	0	0	1.0	–	–
3.5 (ii)	blastocyst	1 (3)	94.4	5.6	0	0.89	–	–
3.5 (iii)	compacted morula	1 (4)	81.2	13.0	5.8	0.74	0.74	53:47
3.5 (iii)	blastocyst	6 (17)	68.8±0.2	21.3±1.2	9.8±0.8	0.57±0.02	0.57±0.03	52:48
4.5 (iii)	hatched blastocyst	10 (28)	52.1±3.9	31.8±2.9	16.1±1.2	0.36±0.06	0.36±0.06	49:51
5.5 (iii)	egg cylinder	2 (2)$^+$	45.1±0.1	43.2±0.5	11.8±0.4	0.14±0.01	0.06±0.02	65:35
5.5 (iv)	egg cylinder	13 (13)	25.3±0.7	47.4±0.3	27.3±0.7	0.05±0.01	0.05±0.01	47:53
6.5 (iv)	egg cylinder	15 (15)	24.8±0.5	48.1±0.6	27.1±0.5	0.04±0.01	0.03±0.01	47:53

* Grouped according to the number of allozyme bands present. Groups iii and iv both have 3 allozymes but in group iv the ratios are close to 25:50:25 (little or no remaining oocyte-coded GPI-1).

** The proportion of oocyte-coded GPI-1 was calculated in two ways. The functions p, q and m were calculated as follows: $q=2x_3/(x_2+2x_3)$; $p=1-q$; $m=(q^2x_1/x_3)-(1-q)^2$. See West *et al* 1986 for derivation of formulae.

$^+$ Individual A:AB:B ratios were 45.2:42.7:12.2 and 45.0:43.6:11.4.

Most of the 5½ and 6½ day samples produced allozymes close to the ratio 1C:4BC:1B but two 6½ day embryos had allozymes that were closer to a ratio of 4C:4BC:1B.

The (B-*Gpi-1s*^{c}×B)F$_1$ embryos produced C, BC and B allozymes rather than A, AB and B. The C allozyme is known to be heat labile *in vitro* (Padua *et al.* 1978) and the stability of the BC allozyme is intermediate between B and C (West and Flockhart, 1989). Although unfertilized eggs from the B-*Gpi-1s*^{c} strain have low GPI-1 activity (Table 1) it has been suggested (West *et al.* 1987) that the activity may be initially higher than normal but declines rapidly *in vivo* to a low level in some tissues. Furthermore, the ratio of C:BC:B allozymes in (B-*Gpi-1s*^{c}×B)F$_1$ hybrids differs from the more usual 1:2:1. We have suggested (West *et al.* 1987) that the initial ratio may be close to 4C:4BC:1B (perhaps as a result of increased synthesis of GPI-1C monomers) and that this 4:4:1 ratio is subsequently modified, in a tissue-specific way, by differential stabilities of the three allozymes. This may account for the 1C:4BC:1B ratios seen in most of the 5½ and 6½ day embryos.

The unusual allozyme ratios, coupled with the differences in stability of the allozymes, introduces some complications to the analysis of the proportion of GPI-1 that is oocyte-coded. Three calculations, based on different assumptions, are shown in Table 5. The most accurate is probably that shown in the last column. This assumes that when no oocyte-coded GPI-1 remains the

Table 5. *Change in GPI-1 allozyme ratios during preimplantation development of (B-*Gpi-1s^c×B)F$_1$ embryos (c/c *females*×b/b *males)*

Age in days *p.c.* (& group*)	Stage	No. of samples (embryos)	Observed percentage of allozyme (Mean±S.E.) C ($100x_1$)	BC ($100x_2$)	B ($100x_3$)	Calculated proportion of oocyte-coded GPI-1** $1\text{-}2x_2$	$1\text{-}(9x_2/4)$	$1\text{-}6x_2/4)$
0.5 (i)	1-cell	11 (54)	100±0	0±0	0±0	1.0	1.0	1.0
0.5 (ii)	1-cell	7 (35)	91.2±0.8	0±0	8.9±0.8^{++}	1.0	1.0	1.0
1.5 (i)	2-cell	8 (39)	100±0	0±0	0±0	1.0	1.0	1.0
2.5 (i)	6- to 12-cell	12 (59)	100±0	0±0	0±0	1.0	1.0	1.0
3.5 (ii)	compacted morula	2 (10)	90.9±0.1	0±0	9.2±0.1^{++}	(1.0)	(1.0)	(1.0)
3.5 (iii)	compacted morula	6 (30)	55.4±3.7	37.9±3.7	6.8±0.7	0.24±0.07	0.15±0.08	0.43±0.06
3.5 (iii)	blastocyst	6 (30)	57.1±3.1	38.1±2.2	4.8±1.0	0.24±0.04	0.14±0.05	0.43±0.03
4.5 (iv)	hatched blastocyst	10 (41)	18.3±1.8	65.2±1.6	16.5±1.1	−0.30±0.03	−0.47±0.04	0.02±0.02
5.5 (iv)	egg cylinder	10 (10)	14.0±1.1	67.2±1.3	18.7±0.5	−0.34±0.03	−0.51±0.03	−0.01±0.02
6.5 (iv)	egg cylinder	23 (23)$^+$	17.9±1.6	63.7±1.4	18.4±0.7	−0.27±0.03	−0.43±0.03	0.04±0.02

* Grouped according to the number of allozyme bands present. Groups iii and iv both have 3 allozymes but in group iv the ratios are close to 17:66:17 (1:4:1).

** The proportion of oocyte-coded GPI-1 was calculated in three ways, assuming that the embryo-coded C:BC:B ratio was 1:2:1, 4:4:1 (theoretical) or 1:4:1 (observed) respectively. See text.

$^+$ Includes three embryos with ratios that differ significantly from 1:4:1. The individaul C:BC:B ratios were 40.4:47.4:12.2, 34.7:57.9:7.4 and 0:84.7:15.3.

$^{++}$ These bands of GPI-1 activity were probably trailing sub-bands of C rather than genuine B bands (see text).

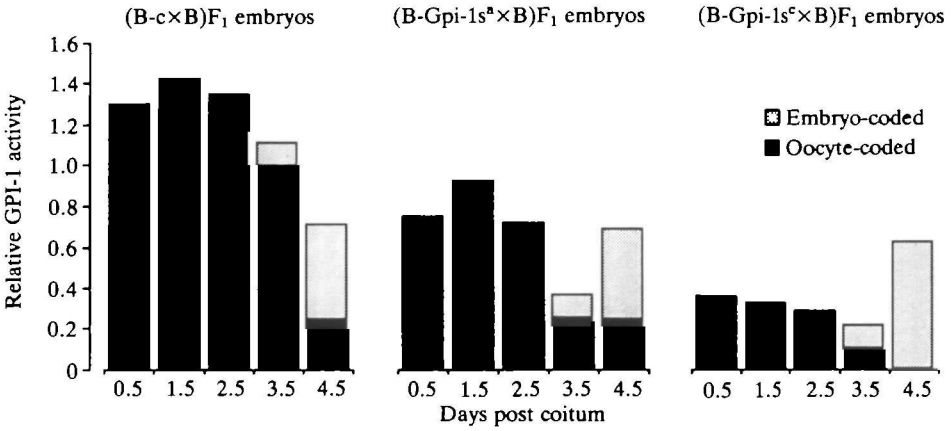

Fig. 2. Loss of oocyte-coded GPI-1 activity and onset of embryo-coded activity in mouse embryos of three different genotypes.

allozyme ratio will be close to 1C:4BC:1B, as seen in most 5½ and 6½ day samples. The proportion of embryo-coded GPI-1 would be the proportion of the BC allozyme multiplied by 6/4; the proportion of oocyte-coded GPI-1 would then be 1 minus the embryo-coded proportion.

Although, in Table 2, the proportion of 3½ day (B-*c*×B)F_1 embryos that had reached the blastocyst stage was lower than those in the other two groups, this was not true for embryos collected for allozyme analysis (Tables 3 to 5). It seems unlikely that there are significant differences in rate of development among the three groups of heterozygous, congenic embryos.

Changes in oocyte-coded and embryo-coded GPI-1 activities in embryos

The calculated proportions of oocyte-coded GPI-1 ($1-2x_2$ in Tables 3 and 4, and $1-(6x_2/4)$ in Table 5) were used to calculate the activities of oocyte-coded and embryo-coded GPI-1 from the total GPI-1 activities shown in Table 2. (Weighted means were used where there were different sub-groups of samples at a particular age. For example, the mean proportion of oocyte-coded GPI-1 in 3½ day (B-*c*×B)F_1 embryos was calculated as 0.90 from the five values (21 samples) given in Table 3.)

The results (Fig. 2) show that in all three groups the embryo-coded GPI-1 is first detected at 3½ days. At this time the oocyte-coded GPI-1 activity differs between the groups but the embryo-coded activity is the same in all groups. By 4½ days, similar levels of oocyte-coded GPI-1 remain in (B-*c*×B)F_1 embryos and (B-*Gpi-1s*^{a}×B)F_1 embryos but this has almost all disappeared from the (B-*Gpi-1s*^{c}×B)F_1 embryos. The total GPI-1 activity is similar in each group because the embryo-coded GPI-1 activity is higher in the (B-*Gpi-1s*^{c}×B)F_1 embryos.

Discussion

The oocyte-coded GPI-1 activity remains at a more or less constant level during the first 2½ days of development in all three genotypes, although this level is different for each genotype. Maintenance of oocyte-coded enzyme levels during the first 2½ days of development suggests either that oocyte-coded GPI-1 enzyme is stable during this phase of development or that enzyme levels are maintained by translation of oocyte-coded GPI-1 mRNA present in the early embryo, as previously discussed for GPI-1 (West *et al.* 1986) and other enzymes (Harper and Monk, 1983). Biochemical and molecular studies suggest that most oocyte-coded mRNA is inactivated in the late 2-cell mouse embryo (Flach *et al.* 1982; Bolton *et al.* 1984) but some mRNA may persist until the early blastocyst stage (Bachvorova and De Leon, 1980).

The oocyte-coded GPI-1 enzyme level begins to fall after 2½ days and is virtually absent by 5½ days in all three genotypes. Some oocyte-coded activity remains in 4½ day (B-*Gpi-1s*^{a}×B)F_1 and (B-*c*×B)F_1 embryos, which both inherit the A allozyme as their oocyte-coded GPI-1, but there is virtually none left in (B-*Gpi-1s*^{c}×B)F_1 embryos, which inherit the more labile C allozyme.

Despite a 4- to 5-fold difference in initial oocyte-coded GPI-1 activity, no differences were seen among the three genotypically distinct groups of embryos in the activation of the embryonic *Gpi-1s* genes. Although embryo-coded GPI-1 was detected in a lower proportion of 3½ day samples of (B-*c*×B)F_1 embryos (11/21) than in the two groups with lower oocyte-coded activity (8/10 and 12/14), both the time when embryo-coded GPI-1 was first detected, and its mean activity at this time, were remarkably consistent in all three groups. Moreover the embryos with the lowest oocyte-coded GPI-1 activity had a form of oocyte-coded GPI-1 that is known to be heat labile *in vitro* and is probably also less stable *in vivo*. The oocyte-coded GPI-1 activity was lower in these embryos, before gene activation, than in the high group once embryo-coded GPI-1 was detected. The similarity, in the timing of embryonic gene expression, between (B-*Gpi-1s*^{c}×B)F_1 embryos and the other two groups implies that the embryonic *Gpi-1s* genes are not activated by a mechanism that depends on a critical threshold of oocyte-coded GPI-1 enzyme activity, but a threshold based on oocyte-coded GPI-1 mRNA remains a possibility.

The occurrence of atypical allozyme ratios suggests that the two embryonic *Gpi-1s*^{a} alleles are not always expressed identically. One 6½ day (B-*c*×B)F_1 embryo

and two 5½ day (B-*Gpi-1s*^{a}×B)F_1 embryos each produced allozymes closer to the ratio 4A:4AB:1B than the typical 1:2:1 ratio (Tables 3 and 4). Residual oocyte-coded GPI-1 or maternal contamination (A allozyme) could explain the high proportion of A allozyme but would not account for the elevated AB/B ratio. It therefore seems more likely that the maternally derived *Gpi-1s*^{a} allele is over-expressed, relative to the paternally derived *Gpi-1s*^{b} allele, in these three atypical embryos. (The expression ratio is approximately 2:1). The overall frequency of this unusual ratio among 5½ and 6½ day embryos of these two genotypes was 3/60 (5%). In all other samples of *Gpi-1s*^{a}/*Gpi-1s*^{b} heterozygous embryos, the expression of the *a* and *b* alleles appeared to be equal. However, as pointed out earlier, the analysis would not detect the expression of the maternally derived, embryo-coded *Gpi-1s* allele until the paternally derived allele is expressed. It is possible that the atypical embryos were either trisomic for chromosome 7 or, more likely, digynic triploids. Takagi and Sasaki (1976) reported a 5-fold increase in the frequency of digynic triploid embryos after A/He strain females were superovulated. At 6½–7½ days, the controls produced 4.5% and the superovulated A/He females produced 19.7% triploid embryos.

Although most studies, like the present one, have first detected embryo-coded GPI-1 in 3½ day morulae or blastocysts, Brinster (1973) detected embryo-coded GPI-1 activity in a group of 500 (SJL/J×C57BL/6)F_1 embryos at the 8-cell stage of development (2½ days). This discrepancy could reflect technical differences, differences in the number of embryos per group, genetic differences between strains of mice that alter the timing of gene expression or, perhaps most likely, the inclusion of precocious embryos (e.g. morulae) among the 2½ day embryos (this could involve genetic differences between strains of mice that affect the rate of development). West *et al.* (1986) also found traces of embryo-coded enzyme in some samples of (129×C57BL)F_1 embryos at 2½ days but these samples included embryos that had advanced beyond the 8-cell stage.

The results of this study answers the two questions originally posed. First, a relatively labile form of oocyte-coded GPI-1 was exhausted before a more stable form of the enzyme. In contrast, the comparison of the high and medium genotypes suggests that the initial GPI-1 activity in the oocyte had relatively little effect on the duration of oocyte-coded activity in these embryos. Second, embryo-coded GPI-1 was first detectable at the same developmental stage (3½ day compacted morulae) in all three groups. The level of oocyte-coded GPI-1 in the high group, when embryo-coded GPI-1 was first detected, was higher than the level in the low group at any stage prior to detection of embryo-coded GPI-1. Thus, the two embryonic *Gpi-1s* alleles cannot be activated by a mechanism that depends on a critical threshold of oocyte-coded GPI-1 enzyme activity. Once both embryonic *Gpi-1s* alleles were activated, they were usually equally expressed in *Gpi-1s*^{a}/*Gpi-1s*^{b} heterozygotes but in a few (possibly digynic triploid) embryos the maternally derived *Gpi-1s* allele was expressed to a greater extent. In conclusion we have revealed a genetic effect on the duration of oocyte-coded enzyme that is probably related to enzyme lability but we have not found a genetic effect on the timing of embryonic gene expression.

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