Developmental profile of glucose phosphate isomerase allozymes in parthenogenetic and tetraploid mouse embryos

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Summary
Glucose phosphate isomerase (GPI) allozymes were compared in eggs and embryos of the mouse strains C57BL/6-JHan (GPI-1BB) and 129/Sv (GPI-1AA) under different experimental conditions. The quantitative differences in eggs of the two strains disappeared by the blastocyst stage at day 4 to 5, both in fertilized and diploid parthenogenetic embryos. The degree of degradation of oocyte-coded enzyme molecules and the activation of the embryonic genome for GPI appeared to be equivalent in parthenogenetic embryos from heterozygous females when only one or other maternal allele type remained in the egg after meiosis. Also in tetraploid embryos, generated by electrofusion of homozygous fertilized eggs from the two strains, both genomes seemed to be activated at the same time at day 4; here, however, the GPI-1BB allozyme remained predominant up to day 6.

Key words: mouse embryos, parthenogenesis, electrofusion, tetraploidy, glucose phosphate isomerase.

Introduction
Early mammalian development is directed by preformed maternal molecules synthesized during oogenesis and by gene products appearing after activation of the embryonic genome (for review see Johnson et al. 1984). Particular attention has been given to the developmental profile of the enzyme glucose phosphate isomerase (GPI) in the mouse. Functional enzyme molecules are synthesized during oogenesis and are present after ovulation (Brinster, 1973; Buehr and McLaren, 1985). This material is used and degraded during preimplantation development (Brinster, 1973), but can still be detected in late blastocysts and early implanting embryos (West and Green, 1983; Duboule and Burki, 1985; Gilbert and Solter, 1985; West et al. 1986; West and Flockhart, 1989a; Petzoldt, 1990). It is however, not clear whether, in addition, mRNA for GPI from oogenesis is present and translated after fertilization (Gilbert and Solter, 1985).

Embryo-coded GPI was first detected between the 8-cell stage (Brinster, 1973) and the morula stage (West and Green, 1983; Duboule and Burki, 1985) when crossing mouse strains homozygous for different GPI allozymes (Chapman et al. 1971). The total amount of active GPI molecules, however, decreases up to the late blastocyst stage (Brinster, 1973; West et al. 1986).

The activity of genes for GPI during mouse oogenesis is controlled by a cis-acting temporal gene (Peterson and Wong, 1978; McLaren and Buehr, 1981). This regulation varies considerably in different mouse strains resulting in varying amounts of active GPI molecules in ovulated eggs obtained from these strains (Peterson et al. 1985). When the embryonic genome is activated, however, these quantitative GPI differences largely disappear by the early egg cylinder stage (West and Flockhart, 1989a) and are not found in somatic cells (Peterson and Wong, 1978).

It was the aim of the present study to compare the loss of oocyte-coded GPI and the increase of embryo-coded GPI in embryos with different levels of oocyte-coded enzyme. For this purpose, we used normal and parthenogenetic embryos from the two mouse strains C57BL/6-JHan (Gpi-1s b/b) and 129/Sv (Gpi-1s a/a) which synthesize defined but different quantities of the enzyme during oogenesis (Peterson et al. 1985). The activity of the enzyme was either quantitatively determined during preimplantation development or the relative amount of the allozymes was compared by coelectrophoresing eggs and embryos of the same age from both strains in the same sample. To compare the loss of maternal molecules and the activation of the different embryonic alleles under similar cellular conditions, either eggs from heterozygous females (129/Sv×C57BL/6-JHan) F1 were used after parthenogenetic activation or homozygous fertilized eggs from both strains were combined by electrofusion techniques.

Materials and methods
Mouse strains, collection and culture of eggs
Mice of the strains 129/Sv (Gpi-1s a/a) and C57BL/6-JHan
(Gpi-l<sup>AA</sup>/b<sup>3</sup>) and heterozygous females (129/Sv×C57BL/6-JHan)F<sub>1</sub> (Gpi-1<sup>AA/b3</sup>) were used for the experiments. To obtain fertilized eggs, females were mated in the afternoon and examined for vaginal plugs the following morning (=day 1 of gestation).

For fusion experiments, eggs were collected at day 1, released from the cumulus masses by treatment with hyaluronidase (Sigma, St. Louis, MO, USA; 150 i.u. ml<sup>-1</sup>) and washed 3 times with Hepes-buffered mouse embryonic culture medium M2 (Fulton and Whittingham, 1978). Cleaving embryos, morulae and blastocysts were flushed from the oviduct or uterus. To remove the zona pellucida, eggs and embryos were treated with pronase (Calbiochem, La Jolla, CA, USA; 3.6 mg ml<sup>-1</sup>) in M2 with polyvinylpyrrolidone (PVP) but without bovine serum albumin (BSA). After several washings in M2, the eggs were directly treated for analysis or transferred into mouse embryoculture medium M16 (Whittingham, 1971) in droplets under paraffin oil (Fisher Sci., Fair Lawn, NJ, USA or British Drug Houses, Poole, UK) and cultured at 37°C in an atmosphere of 5% CO<sub>2</sub> in air or 5% CO<sub>2</sub>, 5% O<sub>2</sub> and 90% N<sub>2</sub>. For <i>in vitro</i> attachment, morulae and blastocysts were transferred to droplets of Eagle’s minimum essential medium (MEM, Flow Laboratories, UK) with 20% fetal calf serum (Monk and Ansell, 1976).

Parthenogenetic activation

Females were superovulated by an intraperitoneal injection of 5 or 7.5 i.u. pregnant mare serum gonadotropin (PMSG, Intergonan, Vemie, Kempen, Germany) and 5 i.u. of human chorionic gonadotropin (hCG, Ekluton, Vemie) administered about 48 h later. Unfertilized eggs were recovered 16 to 17 h post hCG and parthenogenetically activated according to Cuthbertson (1983) using an incubation in M2 with 7% absolute ethanol (Roth, Karlsruhe, Germany) for a period of 7 min. Then eggs were cultured in M16 containing 5 µg ml<sup>-1</sup> cytochalasin B (Aldrich, Milwaukee, MI; USA or Sigma). After removal of the cumulus cells, they were examined for the presence of pronuclei. Only eggs with two pronuclei were selected and further cultured in M16 up to the blastocyst stage.

Electrofusion

After removal of the zona pellucida, single eggs of the strains 129/Sv and C57BL/6-JHan were transferred as pairs to droplets of M16 with phythaemagglutinin (PHA, Sigma; 10 µg ml<sup>-1</sup>) and stored at 37°C for several minutes (Mintz et al. 1973). When they were well agglutinated, they were washed 3 times in M2 and put into a simple fusion chamber (produced in the institute’s workshop) between two platinum wires (200 µm diameter) with a fixed distance apart of 160 µm. Fusion was performed in M2 without BSA with square pulses generated from a dual impedance research stimulator (Harvard; Edenbridge, Kent, UK) connected to an oscilloscope (Rhode and Schwarz, Munich, Germany). Two pulses of 1 kV cm<sup>-1</sup> intensity, a duration of 0.3 ms each and with an interval of about 1 s, were used (Kubiak and Tarkowski, 1985; Kurischko and Berg, 1986; Ozil and Modlinski, 1986). The fused eggs were either directly frozen for analysis or cultured up to day 6.

Enzyme analysis

For GPI analysis, eggs and embryos were washed in M2 without BSA and stored frozen in micropipettes at −20 to −70°C. For photometric determinations only eggs and embryos without zona pellucida were used and stored not longer than 4 days at −70°C (Peterson et al. 1985). After a triple cycle of freezing and thawing, GPI activity was assayed according to Peterson et al. (1985) but using reagents from Boehringer, Mannheim, Germany; Merck, Darmstadt, Germany and Sigma. The reaction was performed in a Kontron Uvicron 860 (Kontron Instruments, Zürich, Switzerland) at 35°C and monitored at 340 nm over a period of 30 min. To calculate the background, each cuvette was pre-run with the reaction mixture but without the sample for 30 min under identical conditions. These values were calculated against a control cuvette containing only the reaction mixture in both determinations.

For electrophoresis, the samples were disrupted by freezing and thawing and directly applied to cellulose acetate plates (Titan III, Helena, Beaumont, TX, USA). After alkyolyme separation and staining according to Eppig et al. (1977), gels were scanned on a Laser Densitometer Ultrascan XL (LKB, Bromma, Sweden). Peaks were cut from the paper, and quantitative estimates were obtained by weight determinations.

Results

For quantitative GPI analysis, eggs and embryos of the strains C57BL/6-JHan (GPI-1BB) and 129/Sv (GPI-1AA) were obtained from the maternal genital tract at defined times. At day 1, both fertilized and/or unfertilized eggs were used in all experiments. Both strains showed developmental variations at day 3 and 4, but differences between the strains were not considered to be significant. Peterson et al. (1985) found that GPI activity was higher in C57BL/6 eggs than in 129/Sv eggs. Fig. 1 shows that this difference remained during cleavage up to the afternoon of day 3 but disappeared at day 4. During this period the total activity of both types of embryo decreased significantly (day 1, 9.00 to day 4, 9.00: GPI-1AA: P=0.01, GPI-1BB: P=<0.001). The GPI activity in parthenogenetically activated and diploidized eggs of both strains was analysed to eliminate paternal effects on GPI activity in developing embryos. Development <i>in vitro</i> is slower and the embryos reached the blastocyst stage at day 5. The development of the 129/Sv parthenotes appeared to lag slightly behind that of C57BL/6 parthenotes. At each day, single embryos from both strains were co-electrophoresed in one sample and the electropherograms were quantitatively evaluated. Fig. 2 shows that there were clear differences of GPI activity between the strains up to day 3 and 4 but these almost disappeared at day 5.

Also determined was the behaviour of the two alleles when expressed together during oogenesis and when only one or the other was activated in developing parthenogenetic diploid embryos. GPI was demonstrated in single embryos up to day 5. During early cleavage, the gels showed the asynchronous pattern of GPI-1AA, 1AB, 1BB derived from oogenesis (Fig. 3). When, in the diploid parthenotes, one or other remaining allele became active, the expression shifted to one of the two homozygotic GPI forms (Fig. 3). Putting all data from days 2 to 5 together, the change of group uniformity for the different GPI forms indicated that the Gpi genes became activated in parthenogenetic
embryos between day 3 and 4 (Fig. 4). A considerable background of oocyte-coded GPI molecules is still visible at day 5 (Fig. 3 and 4).

When one-cell eggs of both strains were combined by electrofusion to form one tetraploid egg, both diploid homozygous genomes were united under equivalent cellular conditions. These eggs were capable of cleavage and reached the early blastocyst stage by day 4 and started to attach to the culture dish at day 6. Using

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**Fig. 1.** Quantitative determinations of GPI activity in eggs and embryos from the mouse strains C57BL/6-JHan (solid line) and 129/Sv (broken line). Activity is given in $A_{	ext{ext}}$ extinction by one egg or embryo per hour. Embryonic stages: day 1 = 1-cell eggs (fertilized or unfertilized); day 2 = 2-cell embryos; day 3, 9.00 = 4- to 8-cell embryos and beginning compaction; day 3, 17.00 = 4-cell stage to compaction; day 4, 9.00 = morulae and blastocysts; day 4, 17.00 = morulae and blastocysts. Bars give the standard deviation; numbers in brackets = number of samples per stage.

**Fig. 2.** Co-electrophoresis of single eggs and diploid parthenogenetic embryos from the strains C57BL/6-JHan (GPI-1BB, solid line) and 129/Sv (GPI-1AA, broken line). GPI activity of the allozymes is given in % of the total activity after densitometric evaluation of the gels. Embryonic stages: day 1 = 1-cell unfertilized eggs; day 2 = 2-cell embryos; day 3 = 4- to 8-cell embryos; day 4 = compaction to morula; day 5 = (expanded) blastocysts. Bars give the standard deviation; numbers in brackets = number of samples per stage.

**Fig. 3.** GPI gels and densitometer curves from diploid parthenogenetic embryos after activation of eggs from heterozygous females (129/SvxC57BL/6-JHan)$F_1$, a and solid line = 2-cell embryo, day 2 showing oocyte-coded GPI; b and broken line = blastocyst, day 5, expressing the $Gpi^F_{1}\beta$ genes; c and dotted line = blastocyst, day 5, expressing the $Gpi^F_{1}\gamma$ genes.
Fig. 4. Electrophoresis of single parthenogenetic embryos after activation of eggs from heterozygous females (129/Sv × C57BL/6-JHan). GPI activity for the 3 different allozymes is given in % of total activity after densitometric evaluation of the gels. Number of samples: day 2 = 9; day 3 = 18; day 4 = 29; day 5 = 10.

Table 1. GPI activity in tetraploid eggs and embryos generated by electrofusion of homozygous fertilized C57BL/6-JHan eggs with homozygous fertilized 129/Sv eggs

<table>
<thead>
<tr>
<th>Age</th>
<th>Stage</th>
<th>No. of embryos (samples)</th>
<th>% GPI-1AA</th>
<th>% GPI-1AB</th>
<th>% GPI-1BB</th>
<th>Ratio of GPI monomers A:Bt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>1-cell</td>
<td>6 (6)</td>
<td>44.6 ± 6.1</td>
<td>–</td>
<td>55.4 ± 6.1</td>
<td>44.6:55.4</td>
</tr>
<tr>
<td>Day 2</td>
<td>2-cell</td>
<td>4 (4)</td>
<td>44.2 ± 8.4</td>
<td>–</td>
<td>55.8 ± 8.4</td>
<td>44.2:55.8</td>
</tr>
<tr>
<td>Day 3</td>
<td>4- to 5-cell</td>
<td>6 (6)</td>
<td>44.0 ± 7.2</td>
<td>–</td>
<td>56.0 ± 7.2</td>
<td>44.0:56.0</td>
</tr>
<tr>
<td>Day 4</td>
<td>Morula to blastocyst</td>
<td>17 (5)</td>
<td>41.3 ± 7.0</td>
<td>7.0 ± 1.1*</td>
<td>51.7 ± 6.1</td>
<td>44.8:55.2</td>
</tr>
<tr>
<td>Day 5</td>
<td>(Expanded) blastocyst</td>
<td>3 (3)</td>
<td>30.9 ± 7.2</td>
<td>22.2 ± 5.6</td>
<td>46.9 ± 4.3</td>
<td>42.0:58.0</td>
</tr>
<tr>
<td>Day 6</td>
<td>Expanded blastocyst, beginning attachment</td>
<td>5 (5)</td>
<td>20.1 ± 6.1</td>
<td>42.6 ± 4.4</td>
<td>37.3 ± 9.2</td>
<td>41.4:58.6</td>
</tr>
</tbody>
</table>

* GPI-1AB peaks were barely visible, electropherograms were approximately evaluated.
† Calculated as GPI-1AA+GPI-1AB/2 to GPI-1BB+GPI-1AB/2 in embryos of days 4 to 6.

Electrophoresis, the first signs of the heteromere GPI-1BB band were visible at day 4, although the results are at the limit of densitometrical evaluation (Table 1). They became stronger at day 5 and 6. Up to day 4 the quantitative correlation between GPI-1AA and GPI-1BB remained unchanged, but at day 5 and 6 this relation was slightly shifted in favour of GPI-1BB (Table 1). As in diploid controls, total GPI activity decreased in tetraploid fusion embryos from the one-cell stage to blastocyst at day 5 (data not shown).

**Discussion**

The results of our study largely confirm data obtained by other groups but using different experimental...
procedures. When taking the quantitative GPI determinations from Peterson et al. (1985), the correlation between the activity of 1.92 in oocytes from the strain C57BL/6-J (GPI-1BB) to that of 1.54 in oocytes from the strain 129/Sv (GPI-1AA) is 55.5 % to 44.5 %. This is very close to our correlations determined after electrophoresis (57.8 % to 42.2 % in Fig. 2 and 55.4 % to 44.6 % in Table 1) as well as to the correlation of $\Delta$ extinction per egg in Fig. 1 (0.0305 to 0.0243 = 55.7 % to 44.3 %). This agreement additionally supports the procedure of West et al. (1989) who normalized data from electrophoresis by using the standard values from Peterson et al. (1985).

The GPI activity during subsequent in vivo development remained at approximately the level of the respective egg up to compaction at day 3 in both strains and declined at the morula to blastocyst stage at day 4. This was also shown by Brinster (1973) and West et al. (1986). By that time, however, the total GPI activity was almost equivalent in both strains, earlier than found by West and Flockhart (1989a). This similar level of activity at the blastocyst stage was also apparent after co-electrophoresis of diploid parthenogenetic eggs and embryos from the two strains, though with some delay after in vitro culture. Obviously, degradation of oocyte-coded GPI and activation of the embryonic genome is regulated in parthenogenetic embryos without a paternal genome in a similar way to that in fertilized eggs.

West and Flockhart (1989a) compared the GPI allozyme relations in heterozygous embryos with different allele combinations and different amounts of oocyte-coded enzyme present. In all cases, the total GPI activity was quite similar at day 5. This was achieved by a relatively slower degradation of oocyte-coded material and a higher activity of the embryonic genome in embryos with a low level of oocyte GPI. The embryonic gene activity, nevertheless, was initiated about the same time. These results might be additionally influenced by different GPI dimer stabilities of the allozyme combinations used (West and Flockhart, 1989b).

Our data from parthenogenetic embryos using eggs from heterozygous females with one or the other maternal allele remaining in diploid form confirm this similar timing of initiation of embryonic gene activity. This is more clearly shown after fusion of homozygous fertilized eggs from the two strains. The GPI-1AB band appeared at day 4, when the ratio of both oocyte-coded allozymes is still the same as after ovulation. The ratio remained, however, in favour of GPI-1BB until the late blastocyst stage with attachment beginning at day 6. This is in contrast to our own results comparing allozyme ratios between homozygous diploid embryos from the two strains (Fig. 1 and 2), but agrees with observations of West et al. (1986), who crossed equivalent mouse strains and analysed the allozyme ratio in heterozygous diploid embryos. They found a monomer ratio between GPI-1A and GPI-1B of 46.5 %:53.5 % in day 7 egg cylinders grown in vivo, a stage almost all GPI was embryo-coded. Therefore, it might be possible, that in heterozygous embryos from these two strains the Gpi-1$s^b$ allele is slightly stronger expressed than the Gpi-1$s^a$ allele. This might be the case also in our heterozygous tetraploid embryos.

In conclusion, our experiments show that in homozygous diploid normal and parthenogenetic embryos the differences in total GPI activity between the strains 129/Sv (GPI-1AA) and C57BL/6-JHan (GPI-1BB) disappeared by the blastocyst stage at day 4 to 5. The embryonic genome was activated in diploid parthenogenetic as well as in tetraploid heterozygous embryos at day 4. In the tetraploid embryos generated by fusion of eggs from the two strains, GPI-1BB activity remained predominant up to day 6. This might be caused by a higher expression of the Gpi-1$s^b$ gene in heterozygous embryos, although we cannot exclude different degradation rates of the oocYTE-coded molecules.

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References


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