A new allele of the *Gpi-1t* temporal gene that regulates the expression of glucose phosphate isomerase in mouse oocytes

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The dimeric enzyme glucose phosphate isomerase (GPI-1) is regulated in oocytes by a *cis*-acting temporal gene (*Gpi-1t*) that maps close to the structural gene (*Gpi-1s*). Quantitative cellulose acetate electrophoresis of GPI-1 allozymes from unfertilized eggs produced by various *Gpi-1s*^{a}/*Gpi-1s*^{b} heterozygous females revealed a new *Gpi-1t* allele that we have designated *Gpi-1t*^{c}. This allele is present in 101/H mice and a partially congenic stock that carries the *Gpi-1s*^{a} gene derived from the AKR strain. We have confirmed that *Gpi-1t*^{c} is closely linked to *Gpi-1s* and that it is *cis*-acting. It produces higher levels of GPI-1 in unfertilized eggs than the other two *Gpi-1t* alleles that are known (*Gpi-1t*^{a} and *Gpi-1t*^{b}) but has no effect on GPI-1 in somatic tissues or spermatozoa. This new *Gpi-1t* allele represents a third developmental programme for GPI-1 expression in oocytes.

1. INTRODUCTION

The dimeric enzyme glucose phosphate isomerase (E.C. 5.3.1.9) is produced by the structural gene *Gpi-1s* (formerly *Gpi-1*) on chromosome 7 of the mouse. In somatic tissues, *Gpi-1s*^{a}/*Gpi-1s*^{b} heterozygotes produce three electrophoretically distinct GPI-1 allozymes in a ratio close to the expected 1 GPI-1A : 2 GPI-1AB : 1 GPI-1B. (These three allozymes represent the AA homodimer, AB heterodimer and BB homodimer respectively).

The GPI-1 activity in unfertilized eggs is a product of gene expression in the diploid oocyte, so that oocytes and unfertilized eggs from heterozygous *Gpi-1s*^{a}/*Gpi-1s*^{b} females also produce three GPI-1 allozymes. Some *Gpi-1s*^{a}/*Gpi-1s*^{b} females produce oocytes and eggs with skewed ratios of allozymes, and this observation led to the discovery of a closely linked temporal locus that regulates the expression of the structural gene in a *cis*-acting, tissue-specific manner (Peterson & Wong, 1978). This temporal locus was initially called *Org* (oocyte regulation of GPI) by Peterson & Wong (1978) but, because the temporal and structural genes appeared to form part of a single gene complex, the gene symbol was changed. Green (1981) suggested replacing the symbol *Org* with *Gpi-1r*, and McLaren & Buehr (1981) suggested using *Gpi-1t*.

According to the present nomenclature (*Mouse News Letter*, **66** (1982), 41) the temporal locus is now designated *Gpi-1t*. Two alleles of *Gpi-1t* are known: DBA/2J

mice are $Gpi-1t^a$ (formerly Org^{low}, $Gpi-1r^l$ or $Gpi-1t^o$) and C57BL/6J mice are $Gpi-1t^b$ (formerly Org^{high}, $Gpi-1r^h$ or $Gpi-1t^s$). Peterson & Wong (1978) showed that unfertilized eggs from DBA/2J mice contained only about 23 % of the GPI-1 activity in C57BL/6J eggs and that eggs from reciprocal F_1 females contained intermediate levels of activity. Quantitative electrophoresis of GPI-1 in eggs from F_1 females ($Gpi-1s^at^a/Gpi-1s^bt^b$) produced skewed allozyme ratios of the order 3–5 % GPI-1A: 30–32 % GPI-1AB: 63–67 % GPI-1B. The ratio of the A and B monomers distributed among the three allozymes was very similar to the ratio of the activities from the homozygotes which suggested that the two $Gpi-1s$ alleles were individually regulated and that the $Gpi-1t$ gene acted *cis*.

Peterson & Wong (1978) found no recombinants between $Gpi-1s$ and Gpi-$1t$ among 65 backcross progeny, thus demonstrating that they were either closely linked on chromosome 7 or were contained within the same locus. Linkage between $Gpi-1s$ and $Gpi-1t$ was supported by McLaren & Buehr (1981), who found 9/41 ($22\cdot0\pm6\cdot5$ %) recombinants between $Gpi-1t$ and c, which is close to the expected $29\cdot1\pm2\cdot7$ % recombination between $Gpi-1s$ and c in females (Davisson & Roderick, 1981).

Although eggs from $Gpi-1s^at^a/Gpi-1s^bt^b$ females produced skewed allozyme ratios Peterson & Wong (1978) reported that red blood cells produced the usual 1:2:1 ratio. McLaren & Buehr (1981) examined the allozyme ratios in maturing oocytes and unfertilized eggs from similar heterozygous females and found that the skewed ratio is first seen in oocytes at 6–7 days after birth.

McLaren & Buehr (1981) cited a personal communication from Peterson claiming that both $Gpi-1s^a$ and $Gpi-1s^b$ structural genes have been found associated with varying degrees of expression at the $Gpi-1t$ locus. However, as far as we know, the only published evidence shows that DBA/2J and MR are homozygous for $Gpi-1s^a$ and $Gpi-1t^a$ and all other inbred strains examined are $Gpi-1t^b$ regardless of the $Gpi-1s$ genotype.

In this paper we report the results of a survey of 13 previously untested strains of mice for new $Gpi-1t$ alleles and new associations between $Gpi-1s$ and $Gpi-1t$ alleles.

2. MATERIALS AND METHODS

Mice, of the strains listed in Table 1 and various F_1 hybrids, were housed under conditions of 16 h light (6 a.m.–10 p.m.) and 8 h dark. Ovulation was induced by intraperitoneal injections of 5 i.u. pregnant mares' serum gonadotrophin, PMS (Folligon, Intervet) at about 4.30 p.m. followed by 5 i.u. human chorionic gonado-trophin, HCG (Chorulon, Intervet) 48 h later.

Mice were killed by cervical dislocation and unfertilized eggs were collected from the oviducts 17–20 h after HCG injection. Cumulus cells were removed from the eggs in a solution containing 100 units of hyaluronidase (Sigma) per ml of phosphate-buffered saline (PBS). The eggs were washed twice in PBS and groups of 3–12 were collected into finely drawn-out Pasteur pipettes, in less than 1 μl of PBS held between two small volumes of paraffin oil, and stored at -20 °C. The samples were frozen and thawed three times in liquid nitrogen vapour, to liberate the enzyme, before analysis.

Spermatozoa were collected from cauda epididymides. Both cauda epididymides were placed in an embryological watch glass, containing 1·5 ml PBS, and cut into small pieces with fine scissors. After allowing at least 5 min for the spermatozoa to swim clear of the tissue, the sperm suspension was drawn into a syringe, through a 22-gauge needle, and transferred to a centrifuge tube. Large tissue fragments were

Table 1. *List of strains of mice used*

Abbreviation	Full strain designation*	Type of strain†	*Gpi-1s*	Strain origin of *Gpi-1s* allele
A	A/He	I	a	
BALB/c	BALB/c ,	I	a	
DBA	DBA/2 Ola/$_e$	I	a	
IS	IS/Cam	I	a	
101	101/H	I	a	
129	129/Sv-*Sl^J-CP*	C	a	129/Sv (?)
B-*Gpi-1s^a*	C57BL/Ola.129 – *Gpi-1s^a*/Ws$_e$	PC (N8)	a	129/Sv-*CP*/Pas-*Fu*^{ki}*Ve*
B-*c*	C57BL/Ola.AKR – *c Gpi-1s^a*/Ws$_e$	PC (N5)	a	AKR/Ola
B10-*p*	C57BL/10-*p*/$_e$	C (N17)	a	NZW
B	C57BL/Ola/$_e$	I	b	
CBA	CBA/H	I	b	
C3H	C3H/HeH	I	b	
JU	JU/FaCt	I	b	
JUN	JU/FaCt-*C*	I, PC (N5F37)	b	JU/FaCt or CBA/Fa
JUW	JU/FaCt-*CA*	I, PC (N5F36)	b	JU/FaCt or CBA/Fa
SM	SM/JH	I	b‡	
TFH	TFH	I	b	

* Subscript e indicates that the strain was introduced to Harwell as pre-implantation embryos transferred to (C3H/HeH ♀ × 101/H ♂)F$_1$ ♀♀.

† I = Inbred, PC = partial congenic, C = congenic, N = number of backcross generations.

‡ Other stocks of SM/J are listed as *Gpi-1s^a* but see Nash (1984).

removed from the suspension by brief centrifugation (a few seconds). Spermatozoa were then collected from the suspension by 5 min centrifugation at 3500 rev./min. The spermatozoa pellet was resuspended in 30 μl of distilled water and stored at −20 °C. The samples were thawed and frozen twice more before analysis and then usually diluted 1:1 with distilled water.

Peripheral blood was collected via heparinized capillary tubes (Gelman Hawksley Ltd) from the orbital sinus, while mice were anaesthetized with ether and stored at −20 °C. Samples of whole blood were usually diluted 1/10 with distilled water before analysis.

The left lateral liver lobe, right kidney, spleen, uterus, intestine, stomach, heart, lungs, thymus, tongue, brain and right hind limb muscle were removed and the stomach and intestine contents washed out in PBS. In addition, gut mesentery, pancreas and mesenteric lymph nodes were either collected as a single sample or divided into two samples: pancreas plus anterior mesentery, and posterior mesentery plus lymph nodes. Each of these samples was homogenized in 1 ml of distilled water using an Ultra-Turrax homogenizer and stored at −20 °C. After thawing, the cell debris was removed by centrifugation for 5 min at 12000 rev./min in a Beckman Microfuge 12 or a MSE Micro Centaur centrifuge. Before electro-

phoresis, the samples were diluted, as appropriate, with distilled water (1/2–1/20 depending on sample).

Samples of cumulus cells were produced as a by-product when unfertilized eggs were collected (see above). Cumulus cells used for analysis were washed twice in PBS and collected into finely drawn-out Pasteur pipettes in PBS, held between two small volumes of paraffin oil. The handling and storage was similar to that described for unfertilized eggs, except that a slightly larger volume of PBS was used and the samples were frozen three times in a $-20\ ^{\circ}$C freezer rather than in liquid nitrogen vapour.

Cellulose acetate electrophoresis was done, using 76×60 mm Helena, Titan III electrophoresis plates, as described by West & Green (1983) except that glycerol was included in the aqueous histochemical staining mixture (32% v/v) to increase the viscosity. Samples were applied to the electrophoresis plates either directly from finely drawn-out Pasteur pipettes (unfertilized eggs, cumulus cells and some spermatozoa samples) or using a $0.25\ \mu l$ Helena Super Z applicator (other somatic tissues and spermatozoa samples). After staining for glucose phosphate isomerase activity, the electrophoresis plates were rinsed in water, fixed for 5 min in 5% acetic acid, washed for 5–10 min in distilled water and dried at room temperature.

The proportions of the different GPI-1 allozymes were quantified by scanning densitometry of the uncleared, dried electrophoresis plates using a Helena Auto Scanner Flur-Vis densitometer fitted with a 545 nm filter.

Electrophoresis of the spermatozoa samples in experiment 1 (Table 7) was done without adding glycerol to the staining mixture and the allozymes were quantified using a Gelman DCD-16 computing densitometer as described by West & Green (1983). Mice used in this experiment were housed in the Zoology Department of the University of Oxford.

3. RESULTS

(i) *Screen of inbred and congenic stocks of mice*

Six previously untested Gpi-$1s^{a}$ strains of mice and seven Gpi-$1s^{b}$ strains were screened for variants of the temporal Gpi-$1t$ locus by quantitative electrophoresis of the GPI-1 allozymes present in unfertilized eggs from adult heterozygous, Gpi-$1s^{a}/Gpi$-$1s^{b}$ female progeny.

It was previously established that the DBA strain carries Gpi-$1t^{a}$ and that B and B-Gpi-$1s^{a}$ strains carry Gpi-$1t^{b}$ (Peterson & Wong, 1978; West & Green, 1983). This was confirmed by two pairs of reciprocal crosses (8, 21, 1 and 9) shown in Table 2.

The F_1 female progeny, of crosses between females from three of the Gps-$1s^{a}$ strains (A, BALB/c and IS) and B males, produced eggs with allozyme ratios close to 25% GPI-1A:50% GPI-1AB:25% GPI-1B (crosses 2–4 in Table 2). The F_1 female offspring, of crosses between all seven of the Gpi-$1s^{b}$ strains tested (CBA, C3H, JU, JUN, JUW, SM and TFH) and B-Gpi-$1s^{a}$ males, also produced eggs with a similar pattern of GPI-1 allozymes. Although in most cases there was a slight excess of GPI-1B monomers the results indicate that both parents of these F_1 females carry the same Gpi-$1t$ allele. Therefore these ten strains carry the Gpi-$1t^{b}$ allele.

Table 2. *Quantitative analysis of GPI-1 allozymes in unfertilized eggs from*
Gpi-1s^a/Gpi-1s^b *heterozygous females to screen for* Gpi-1t *variants*

Cross no.	Origin of heterozygous females ($♀ × ♂$)	No. of samples	Total no. of eggs	Mean % of allozymes ($\pm$ s.e.)			Estimated A:B monomer ratio ($p:q$)*
				A ($100\,x_1$)	AB ($100\,x_2$)	B ($100\,x_3$)	
	I. Screen of *Gpi-1s*^{a} stocks						
1	B-*Gpi-1s*^{a} × B	12	97	21·3 ± 0·6	48·7 ± 0·6	30·1 ± 0·2	0·46:0·54
2	A × B	13	90	23·8 ± 0·7	50·4 ± 0·3	25·8 ± 0·7	0·49:0·51
3	BALB/c × B	15	86	19·9 ± 0·6	53·1 ± 0·8	26·9 ± 1·2	0·47:0·54
4	IS × B	7	40	22·6 ± 0·8	50·8 ± 1·0	26·6 ± 1·3	0·48:0·52
5	101 × B	10	57	48·1 ± 1·2	45·2 ± 1·1	6·7 ± 0·8	0·71:0·29
6	B-c × B†	9	76	40·5 ± 0·5	49·5 ± 0·9	9·9 ± 0·8	0·65:0·35
7	B10-*p* × B	16	120	10·0 ± 0·4	38·7 ± 0·4	51·3 ± 0·6	0·29:0·71
8	DBA × B	5	44	9·3 ± 0·5	38·8 ± 0·3	51·9 ± 0·3	0·29:0·71
	II. Screen of *Gpi-1s*^{b} stocks						
9	B × B-*Gpi-1s*^{a}	17	94	21·9 ± 0·6	51·9 ± 0·8	26·1 ± 0·5	0·48:0·52
10	CBA × B-*Gpi-1s*^{a}	8	60	22·9 ± 0·5	48·6 ± 0·7	28·5 ± 0·6	0·47:0·53
11	C3H × B-*Gpi-1s*^{a}	11	67	22·4 ± 0·6	48·9 ± 0·8	28·4 ± 0·7	0·47:0·53
12	JU × B-*Gpi-1s*^{a}	9	66	21·3 ± 0·6	54·3 ± 0·5	24·4 ± 0·8	0·48:0·52
13	JUN × B-*Gpi-1s*^{a}	14	78	22·9 ± 0·5	48·3 ± 0·6	28·9 ± 0·4	0·47:0·53
14	JUW × B-*Gpi-1s*^{a}	6	34	21·4 ± 0·2	53·6 ± 0·4	25·0 ± 0·6	0·48:0·52
15	SM × B-*Gpi-1s*^{a}	10	72	24·2 ± 0·7	52·3 ± 0·8	23·5 ± 0·4	0·50:0·50
16	TFH × B-*Gpi-1s*^{a}	9	52	20·3 ± 0·6	53·2 ± 0·6	26·5 ± 1·1	0·47:0·53
	III. Reciprocal and other crosses						
17	B × 101	17	134	43·7 ± 0·4	44·7 ± 0·3	11·6 ± 0·4	0·66:0·34
18	C3H × 101	12	42	48·0 ± 1·2	44·1 ± 1·1	8·0 ± 0·6	0·70:0·30
19	B × B-c†	7	52	41·3 ± 0·8	47·2 ± 0·9	11·5 ± 0·4	0·65:0·35
20	B × B10-*p*	8	69	5·6 ± 0·2	33·8 ± 0·4	60·7 ± 0·4	0·22:0·78
21	B × DBA	11	93	9·7 ± 0·2	38·8 ± 0·4	51·5 ± 0·5	0·29:0·71

* $p = x_1 + (x_2/2)$; $q = x_3 + (x_2/2)$.
† B-c mice were *Gpi-1s*^{a}/*Gpi-1s*^{b} heterozygous backcross progeny produced while making the partially congenic strain.

Unfertilized eggs, produced by F_1 females from reciprocal matings between B10-*p* and B (crosses 7 and 20 in Table 2), had skewed allozyme ratios that were similar to those produced by eggs from *Gpi-1s*^{a}*t*^{a}/*Gpi-1s*^{b}*t*^{b} progeny of reciprocal DBA × B matings (crosses 8 and 21). We therefore conclude that the B10-*p* strain carries the *Gpi-1t*^{a} allele. The parent B10 strain is *Gpi-1s*^{b} so the *Gpi-1s*^{a} allele in B10-*p* must have originated from the NZW strain along with the linked *p* allele (recombination frequency = 16·2 ± 3·1 %, Roderick & Davisson, 1981). NZW must therefore also carry *Gpi-1t*^{a}.

Eggs from F_1 female progeny of crosses between 101 or B-c and B or C3H mice (crosses 5, 6, 17–19) all produced a third pattern of GPI-1 allozymes. This showed skewing in the opposite direction from those produced by eggs from *Gpi-1s*^{a}*t*^{a}/*Gpi-1s*^{b}*t*^{b} females. It seemed likely that 101 and B-c mice carried a third *Gpi-1t* allele, that acted in *cis* on *Gpi-1s*^{a}, and caused an elevated production of GPI-1A monomers in oocytes. Since the *Gpi-1s*^{a} allele in B-c mice came from the AKR strain, this strain would also carry this new allele.

In order to test whether this skewed allozyme pattern is caused by a new *Gpi-1t*

allele we have investigated the impact of this variant on GPI-1 in eggs produced by $Gpi-1s^a/Gpi-1s^b$ heterozygotes and $Gpi-1s^a/Gpi-1s^a$ homozygotes and tested the tissue specificity and genetic linkage of the skewed allozyme ratio. For convenience, the variant is provisionally designated $Gpi-1t^c$ at this point.

(ii) *The impact of the new $Gpi-1t^c$ allele on GPI-1 in unfertilized eggs*

The effect of heterozygosity for $Gpi-1t$ on GPI-1 allozymes in eggs from $Gpi-1s^a/Gpi-1s^b$ females has been described above and is illustrated in Plate 1, Fig. 1.

Altered allozyme ratios could be produced in many ways. The two types of monomers could be produced at different rates, at different times, have different stabilities or, for some other reason, may dimerize non-randomly. Alternatively the dimers may be formed in a $1:2:1$ ratio which is then altered by differential stability of the dimers. Peterson & Wong (1978) argued against this last possibility for $Gpi-1s^a t^a/Gpi-1s^b t^b$ heterozygotes.

The analysis of the allozyme ratios, shown in Table 3, gives an indication that this is also unlikely to be the cause of the skewing seen in eggs from $Gpi-1s^a t^c/Gpi-1s^b t^b$ heterozygotes. The proportions of the A and B monomers present in the dimers at the time of analysis are shown as p and q respectively. If the skewing was a result of unequal monomer production (or unequal monomer degradation) then these values of p and q should represent the proportions of monomers available to form dimers. If the monomers aggregate at random the expected allozyme ratio is $p^2:2pq:q^2$. These predicted ratios are shown in Table 3 and are close to the observed allozyme ratios for all of the crosses.

This is not true, for example, for the skewed allozyme ratios produced by heterozygosity for the $Gpi-1s^c$ structural allele. Here skewing is a result of differential instability of the GPI-1C allozyme. West & Green (1983) reported an allozyme ratio of 32 % B:68 % BC:0 % C in unfertilized eggs for $Gpi-1s^b/Gpi-1s^c$ females. The expected ratio, based on monomer proportions of 0·66 B and 0·34 C, is 44 % B:49 % BC:12 % C.

The concordance between the observed and expected allozyme ratios shown in Table 3 argues that the skewing is present before dimer formation and that A and B monomers are produced synchronously and dimerize at random. However, alone, this is a weak argument because it is still possible that differences in stability of all three dimers could, by chance, produce the expected ratios.

One of Peterson & Wong's main arguments was based on the similarity between the ratio of GPI-1 monomers in eggs from heterozygous females and the ratio of GPI-1 activities in eggs of the two homozygous, parental strains (Peterson & Wong, 1978). Similarly, the $p:q$ ratios shown in Table 3 provide an estimate of the ratio of GPI activities in eggs from parental strains that is expected if the skewing is a result of differential monomer production or degradation.

The $Gpi-1s^b$ allele is associated with the $Gpi-1t^b$ allele in all of the crosses shown in Table 3 and is derived from the same strain in 10 out of the 11 crosses. If there is *cis*-acting regulation of monomer production or degradation, the expression of B monomers should be the same in all of the crosses. In Table 3 the proportion
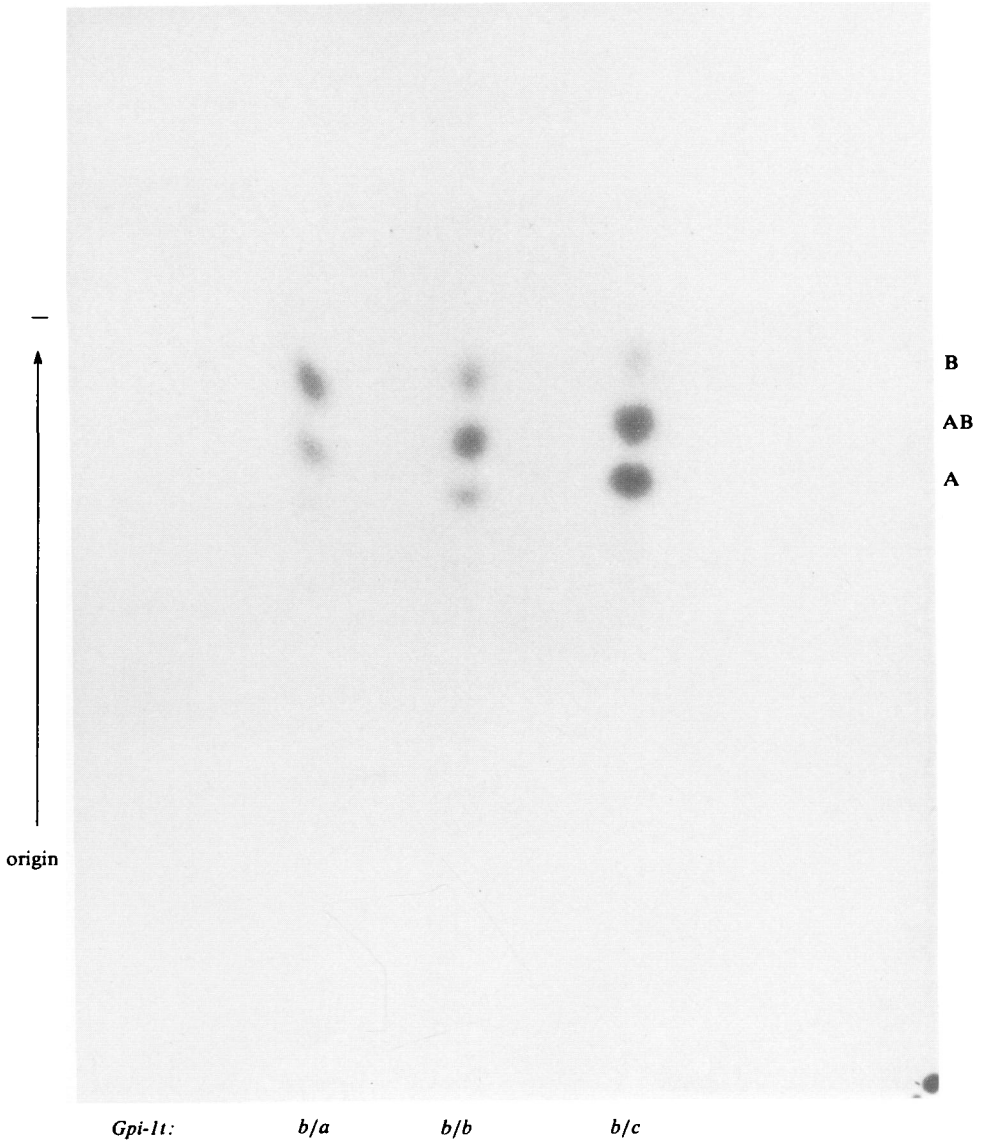
Fig. 1. GPI-1 allozymes (labelled A, AB and B) produced by five unfertilized eggs from each of three heterozygous $Gpi\text{-}1s^{b}/Gpi\text{-}1s^{a}$ females. $Gpi\text{-}1t$ genotypes are shown as b/a, b/b and b/c. $b/a = (\text{B} \times \text{DBA})\text{F}_1 \female$; $b/b = (\text{B} \times \text{B-}Gpi\text{-}1s^{a})\text{F}_1 \female$; $b/c = (\text{B} \times 101)\text{F}_1 \female$.

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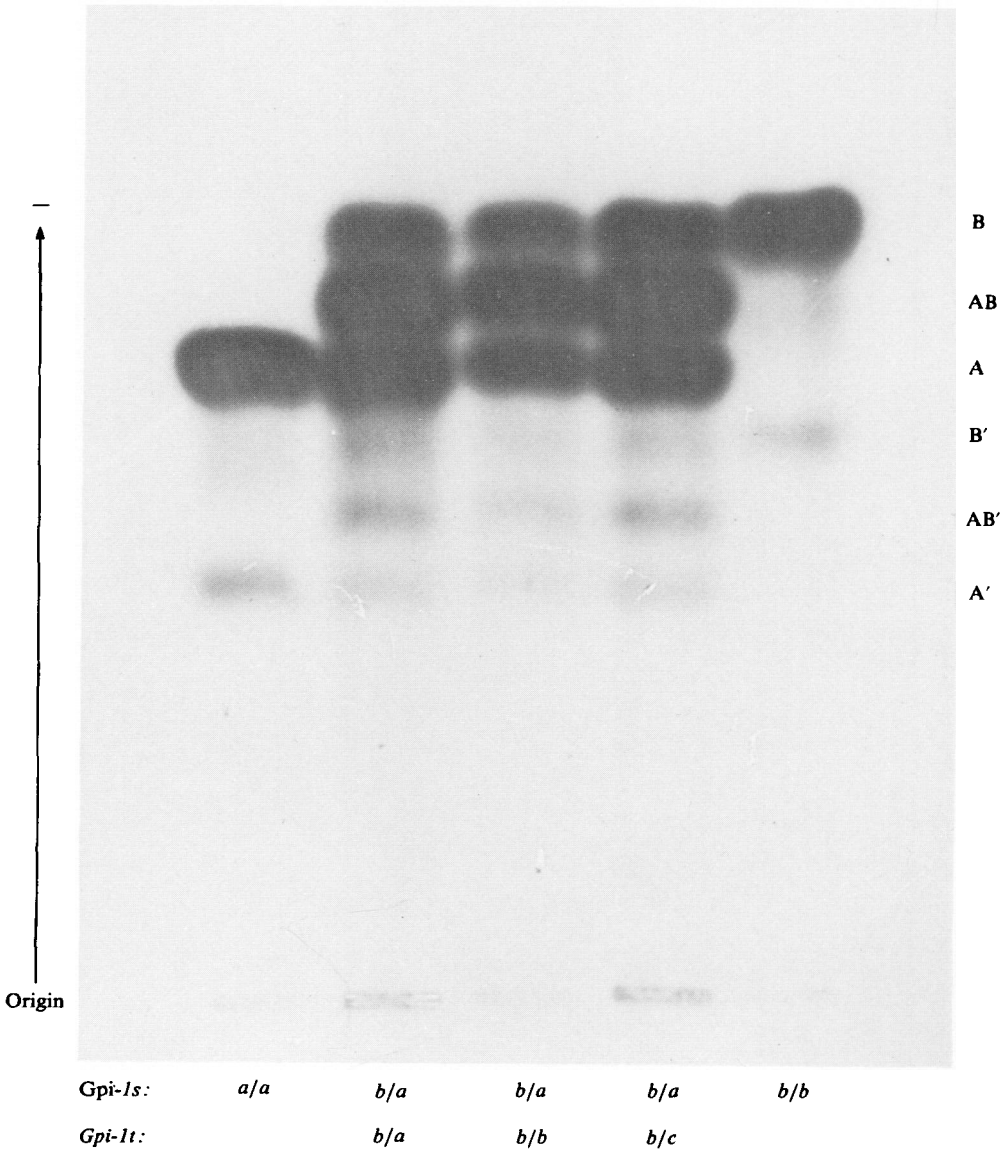Fig. 2. GPI-1 allozymes produced by epididymal spermatozoa samples from five mice. The three major GPI-1 allozymes are labelled A, AB and B and the three minor allozymes are marked A′, AB′ and B′. The three *Gpi-1s*^{b}/*Gpi-1s*^{a} heterozygotes had different *Gpi-1t* genotypes: $b/a = (B \times DBA)F_1 \male$; $b/b = (B \times B\text{-}Gpi\text{-}1s^a)F_1 \male$; $b/c = (B \times 101)F_1 \male$.

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of the A monomer is calculated relative to the B monomer for the various crosses. The predicted ratios of GPI-1 activity in eggs from females homozygous for $Gpi\text{-}1s^a t^a$, $Gpi\text{-}1s^a t^b$ and $Gpi\text{-}1s^a t^c$ lies between the most extreme and the most conservative ratios that can be constructed from the values of the relative expression of A monomer for $Gpi\text{-}1t^a$, $Gpi\text{-}1t^b$ and $Gpi\text{-}1t^c$ (Table 3). The most extreme ratio is $1:3:8\cdot7$ and the most conservative ratio is $1\cdot4:3:6\cdot6$ (or $1:2\cdot1:4\cdot7$). The lack of precision in this prediction is caused by the variability among allozyme ratios.

Table 3. *The effect of the three* Gpi-1t *alleles on GPI-1 allozyme ratios in unfertilized eggs from some of the* Gpi-1s^a/Gpi-1s^b *females shown in Table 2*

Cross no.	*Gpi-1t* genotype	Observed mean % of allozymes, A:AB:B	Estimated A:Bmonomer ratio $(p:q)$	Relative expression of A monomer*	Expected ratios of allozymes (%), $100\ p^2 : 200\ pq : 100\ q^2$
20	*b/a*	$5\cdot6:33\cdot8:60\cdot7$	$0\cdot22:0\cdot78$	$1\cdot0$	$5:34:61$
7	*a/b*	$10\cdot0:38\cdot7:51\cdot3$	$0\cdot29:0\cdot71$	$1\cdot4$	$8:41:50$
21	*b/a*	$9\cdot7:38\cdot8:51\cdot5$	$0\cdot29:0\cdot71$	$1\cdot4$	$8:41:50$
8	*a/b*	$9\cdot3:38\cdot8:51\cdot9$	$0\cdot29:0\cdot71$	$1\cdot4$	$8:41:50$
1	*b/b*	$21\cdot3:48\cdot7:30\cdot1$	$0\cdot46:0\cdot54$	$3\cdot0$	$21:50:29$
9	*b/b*	$21\cdot9:51\cdot9:26\cdot1$	$0\cdot48:0\cdot52$	$3\cdot3$	$23:50:27$
19	*b/c*	$41\cdot3:47\cdot2:11\cdot5$	$0\cdot65:0\cdot35$	$6\cdot6$	$42:46:12$
6	*c/b*	$40\cdot5:49\cdot5:9\cdot9$	$0\cdot65:0\cdot35$	$6\cdot6$	$42:46:12$
17	*b/c*	$43\cdot5:44\cdot7:11\cdot6$	$0\cdot66:0\cdot34$	$6\cdot9$	$44:49:12$
18	*b/c*	$48\cdot0:44\cdot1:8\cdot0$	$0\cdot70:0\cdot30$	$8\cdot3$	$49:42:9$
5	*c/b*	$48\cdot1:45\cdot2:6\cdot7$	$0\cdot71:0\cdot29$	$8\cdot7$	$50:41:8$

* The relative expression of the A monomer in the different heterozygotes is calculated, assuming constant expression of the B monomer, as p/q. These values are normalized by dividing by the p/q value for cross 20.

Plate 1, Fig. 1 provides pictorial evidence that the total GPI-1 activity in eggs from $Gpi\text{-}1s^b/Gpi\text{-}1s^a$ heterozygotes increases in the order $Gpi\text{-}1t^b/Gpi\text{-}1t^a < Gpi\text{-}1t^b/Gpi\text{-}1t^b < Gpi\text{-}1t^b/Gpi\text{-}1t^c$ since the same number of eggs was used in each track. Estimates of the ratio of GPI-1 activities, in eggs from females that were all homozygous, $Gpi\text{-}1s^a/Gpi\text{-}1s^a$ but homozygous for different $Gpi\text{-}1t$ temporal alleles, were obtained by scanning across the (GPI–1A) allozymes produced by eggs run in three adjacent tracks of an electrophoresis plate. The results (Table 4) show that GPI-1 activity increases in the order $Gpi\text{-}1t^a/Gpi\text{-}1t^a < Gpi\text{-}1t^b/Gpi\text{-}1t^b < Gpi\text{-}1t^c/Gpi\text{-}1t^c$. Moreover the mean ratio ($10\cdot8\ \% : 28\cdot7\ \% : 60\cdot8\ \% = 1:2\cdot7:5\cdot6$) is within the range predicted above (between $1:2\cdot1:4\cdot7$ and $1:3:8\cdot7$).

The ratio of GPI-1 activities produced by eggs from $Gpi\text{-}1s^a t^a/Gpi\text{-}1s^a t^a$ and $Gpi\text{-}1s^a t^b/Gpi\text{-}1s^a t^b$ homozygotes (calculated as $0\cdot27:0\cdot73$ from Table 4) is also in good agreement with ratios derived from spectrophotometric assays of GPI-1 in eggs from $Gpi\text{-}1s^a t^a/Gpi\text{-}1s^a t^a$ and $Gpi\text{-}1s^b t^b/Gpi\text{-}1s^b t^b$ females ($0\cdot18:0\cdot82$, Peterson & Wong, 1978; $0\cdot28:0\cdot72$, McLaren & Buehr, 1981).

The agreement between the ratios predicted by Table 3 and those shown in Table 4 shows that the new variant, provisionally designated $Gpi\text{-}1t^c$, does behave like an allele of the $Gpi\text{-}1t$ temporal locus. It appears to control the production of GPI-1 in eggs by regulating the monomer production or degradation prior to dimer formation.

Table 4. *Relative GPI-1 activity in unfertilized eggs from* Gpi-1s^a/Gpi-1s^a *homozygous females estimated by quantitative electrophoresis*

Number of eggs	No. of samples	Mean staining ratio ($\pm$ s.e.)					Mean specific staining ratio†				
a:b:c*		a	:	b	:	c	a	:	b	:	c
3:3:3	7	11.0 ± 0.6		29.5 ± 1.0		59.7 ± 1.1	11.0 ± 0.6		29.5 ± 1.0		59.7 ± 1.1
8:3:1	3	36.1 ± 0.8		35.7 ± 1.0		28.2 ± 0.7	10.1 ± 0.3		26.7 ± 0.9		63.1 ± 0.9
Total	10						10.8 ± 0.4		28.7 ± 0.8		60.8 ± 0.9

* a = DBA eggs (*Gpi-1t^a/Gpi-1t^a*), b = B-*Gpi-1s^a* eggs (*Gpi-1t^b/Gpi-1t^b*), c = 101 strain eggs (*Gpi-1t^c/Gpi-1t^c*).

† Specific staining ratio is adjusted to allow for unequal numbers of eggs.

(iii) *Genetic linkage of Gpi-1t^c*

The linkage experiment shown in Table 5 was undertaken to test whether our new variant was closely linked to the *Gpi-1s* structural locus. Male offspring were discarded and females were typed for albino (*c*) and *Gpi-1s*, using blood samples. Female progeny were distributed among four classes as follows: 45 *Gpi-1s^a + / Gpi-1s^b c*; 50 *Gpi-1s^b c/Gpi-1s^b c* (parental types); 29 *Gpi-1s^b + /Gpi-1s^b c*; 13 *Gpi-1s^a c/Gpi-1s^b c* (recombinants). Overall the recombinant fraction between *Gpi-1s* and *c* was 42/137 ($30.7 \pm 3.9\%$) which is in good agreement with the published figure of $29.1 \pm 2.7\%$ recombination in females (Davisson & Roderick, 1981).

Unfertilized eggs were collected from 50 of the *Gpi-1s^a/Gpi-1s^b* females in order to type them for *Gpi-1t*. All 50 females were successfully typed for *Gpi-1t* by visual assessment of the allozyme ratio and, as shown in Table 5, all of those tested produced skewed allozyme ratios typical of the parental *Gpi-1s^{a}t^c/Gpi-1s^{b}t^b* genotype. This confirms that the new *Gpi-1t^c* variant is closely linked to the *Gpi-1s* structural locus on chromosome 7. Peterson & Wong (1978) found no recombinants between *Gpi-1t^a* and *Gpi-1s^a* among 65 progeny. Using Simon's formula (cited by Dizik & Elliot, 1977) on the pooled data (0/115 recombinants), it is calculated that the 95% confidence limits place those two loci within 2.6 map units of each other.

(iv) *Tissue specificity*

Visual assessment of GPI-1 allozyme ratios suggests that *Gpi-1t^c* has no effect in various somatic tissues of *Gpi-1s^{a}t^c/Gpi-1s^{b}t^b* mice. The 50 mice in the linkage experiment that produced eggs with skewed allozyme ratios all showed ratios that were close to 1:2:1 for blood GPI-1. Similarly two samples of cumulus cells from each of three of these females produced ratios close to 1:2:1.

Typical 1:2:1 ratios were again seen in 13 samples of somatic tissues or organs (liver, kidney, spleen, uterus, intestine, stomach, heart, lungs, thymus, brain, tongue, limb muscle and whole blood) from two *Gpi-1s^{b}t^b/Gpi-1s^{a}t^c* and one *Gpi-1s^{b}t^b/Gpi-1s^{a}t^a* females ((B × 101) F$_1$ and (B × DBA) F$_1$ respectively). Pancreas and gut mesentery samples from all three mice, however, produced relatively weak GPI-1B allozymes.

Table 5. *Recombination between* Gpi-1s, Gpi-1t *and* c *among heterozygous* Gpi-1s^a/Gpi-1s^b *female progeny*

Cross: (101 ♀ × JU ♂)F$_1$ ♀ × JU ♂

$$\frac{\text{Gpi-}1s^a t^c +}{\text{Gpi-}1s^b t^b c} \times \frac{\text{Gpi-}1s^b t^b c}{Gpi\text{-}1s^b t^b c}$$

Gpi-1s^a/Gpi-1s^b progeny

Parental genotype:	$s^a t^c + /s^b t^b c$	40
Recombinant genotypes:	$s^a t^c c /s^b t^b c$	10
	$s^a t^b c /s^b t^b c$	0
	$s^a t^b + /s^b t^b c$	0

Percent recombination

Gpi-1s – Gpi-1t	0/50	0 %
Gpi-1s – c	10/50	$20 \cdot 0 \pm 5 \cdot 7$ %
Gpi-1t – c	10/50	$20 \cdot 0 \pm 5 \cdot 7$ %

Table 6. *Quantitative analysis of GPI-I allozymes in somatic tissues of* Gpi-1s^b/Gpi-1s^a *heterozygous females*

Organ or tissue	*Gpi-1t* genotype*	Mean % of allozymes (±S.E.)			Estimated A:B monomer ratio
		A	AB	B	
Whole blood	*b/a*	$29 \cdot 1 \pm 0 \cdot 5$	$48 \cdot 0 \pm 0 \cdot 3$	$22 \cdot 9 \pm 0 \cdot 2$	$0 \cdot 53 : 0 \cdot 47$
	b/b	$31 \cdot 9 \pm 0 \cdot 3$	$48 \cdot 7 \pm 0 \cdot 2$	$19 \cdot 5 \pm 0 \cdot 4$	$0 \cdot 56 : 0 \cdot 44$
	b/c	$28 \cdot 1 \pm 0 \cdot 4$	$44 \cdot 2 \pm 0 \cdot 3$	$27 \cdot 7 \pm 0 \cdot 1$	$0 \cdot 50 : 0 \cdot 50$
Brain	*b/a*	$30 \cdot 1 \pm 0 \cdot 5$	$47 \cdot 3 \pm 0 \cdot 6$	$22 \cdot 7 \pm 0 \cdot 7$	$0 \cdot 54 : 0 \cdot 46$
	b/b	$30 \cdot 7 \pm 0 \cdot 5$	$48 \cdot 5 \pm 0 \cdot 8$	$20 \cdot 8 \pm 0 \cdot 5$	$0 \cdot 55 : 0 \cdot 45$
	b/c	$32 \cdot 5 \pm 0 \cdot 5$	$45 \cdot 9 \pm 0 \cdot 8$	$21 \cdot 6 \pm 0 \cdot 6$	$0 \cdot 55 : 0 \cdot 45$
Liver	*b/a*	$32 \cdot 2 \pm 0 \cdot 8$	$46 \cdot 7 \pm 1 \cdot 1$	$21 \cdot 1 \pm 0 \cdot 6$	$0 \cdot 56 : 0 \cdot 44$
	b/b	$30 \cdot 4 \pm 0 \cdot 4$	$43 \cdot 8 \pm 0 \cdot 4$	$25 \cdot 9 \pm 0 \cdot 8$	$0 \cdot 52 : 0 \cdot 48$
	b/c	$33 \cdot 9 \pm 0 \cdot 7$	$42 \cdot 2 \pm 0 \cdot 1$	$24 \cdot 0 \pm 0 \cdot 7$	$0 \cdot 55 : 0 \cdot 45$
Kidney	*b/a*	$20 \cdot 9 \pm 0 \cdot 5$	$49 \cdot 8 \pm 0 \cdot 7$	$29 \cdot 3 \pm 0 \cdot 8$	$0 \cdot 46 : 0 \cdot 54$
	b/b	$30 \cdot 3 \pm 0 \cdot 5$	$46 \cdot 8 \pm 0 \cdot 3$	$22 \cdot 9 \pm 0 \cdot 5$	$0 \cdot 54 : 0 \cdot 46$
	b/c	$29 \cdot 3 \pm 0 \cdot 2$	$44 \cdot 6 \pm 0 \cdot 4$	$26 \cdot 2 \pm 0 \cdot 4$	$0 \cdot 52 : 0 \cdot 49$
Pancreas†	*b/a*	$37 \cdot 4 \pm 0 \cdot 3$	$43 \cdot 6 \pm 0 \cdot 6$	$18 \cdot 9 \pm 0 \cdot 7$	$0 \cdot 59 : 0 \cdot 41$
	b/b	$35 \cdot 7 \pm 0 \cdot 5$	$46 \cdot 8 \pm 0 \cdot 7$	$17 \cdot 5 \pm 0 \cdot 5$	$0 \cdot 59 : 0 \cdot 41$
	b/c	$37 \cdot 1 \pm 1 \cdot 1$	$45 \cdot 8 \pm 0 \cdot 7$	$17 \cdot 2 \pm 1 \cdot 7$	$0 \cdot 60 : 0 \cdot 40$
Mesentery†	*b/a*	$32 \cdot 2 \pm 1 \cdot 2$	$45 \cdot 9 \pm 0 \cdot 8$	$22 \cdot 0 \pm 0 \cdot 8$	$0 \cdot 55 : 0 \cdot 45$
	b/b	$33 \cdot 5 \pm 0 \cdot 6$	$43 \cdot 5 \pm 0 \cdot 4$	$23 \cdot 0 \pm 0 \cdot 5$	$0 \cdot 55 : 0 \cdot 45$
	b/c	$33 \cdot 8 \pm 0 \cdot 7$	$46 \cdot 7 \pm 1 \cdot 1$	$19 \cdot 5 \pm 0 \cdot 8$	$0 \cdot 57 : 0 \cdot 43$

* Six females of each genotype were used for all of the samples analysed: $b/a = $ (B × DBA)F$_1$ ♀♀; $b/b = $ (B × B-*Gpi-1s^a*)F$_1$ ♀♀; $b/c = $ (B × 101)F$_1$ ♀♀.

† Pancreas sample includes some anterior gut mesentery. Mesentery sample is posterior gut mesentery and includes the mesenteric lymph nodes.

The allozyme ratios produced by somatic tissues of *Gpi-1s^b/Gpi-1s^a* heterozygotes of three different *Gpi-1t* genotypes were investigated quantitatively for six tissues and organs, including the pancreas and mesentery (Table 6). In contrast to the results for unfertilized eggs from *Gpi-1s^{a}t^b/Gpi-1s^{b}t^b* females (Table 2), the somatic tissues tended to have a slight excess of the GPI-1A monomer. The results for whole blood, brain and liver showed little difference between the three *Gpi-1t* genotypes, and all samples produced ratios reasonably close to $1 : 2 : 1$. This was also true of the kidney samples from *Gpi-1t^b/Gpi-1t^b* and *Gpi-1t^b/Gpi-1t^c* mice, but

kidney from $Gpi\text{-}1t^b/Gpi\text{-}1t^a$ mice produced a slight excess of GPI-1B monomer rather than GPI-1A. (Interestingly, Padua, Bulfield & Peters (1978) reported slightly higher GPI-1 activities in erythrocytes, brain and liver from $Gpi\text{-}1s^a/Gpi\text{-}1s^a$ homozygotes than from $Gpi\text{-}1s^b/Gpi\text{-}1s^b$ homozygotes, but in kidney this ratio was reversed.)

Table 7. *Quantitative analysis of GPI-1 allozymes in epididymal spermatozoa of* Gpi-1s^a/Gpi-1s^b *heterozygous males*

GPI-1 allozyme set*	*Gpi-1t* genotype†	No. of samples	Mean % of allozymes ($\pm$ S.E.)			Estimated A:B monomer ratio
			A	AB	B	
			Experiment 1			
Major	a/b	5	$28\cdot6\pm0\cdot8$	$46\cdot6\pm1\cdot0$	$24\cdot8\pm0\cdot3$	$0\cdot52:0\cdot48$
	b/b	5	$30\cdot5\pm0\cdot4$	$43\cdot5\pm0\cdot8$	$26\cdot1\pm0\cdot5$	$0\cdot52:0\cdot48$
Minor	a/b	5	$23\cdot1\pm1\cdot5$	$48\cdot0\pm2\cdot1$	$28\cdot9\pm2\cdot2$	$0\cdot47:0\cdot53$
	b/b	5	$27\cdot4\pm0\cdot6$	$49\cdot3\pm1\cdot0$	$23\cdot2\pm0\cdot6$	$0\cdot52:0\cdot48$
			Experiment 2‡			
Major	b/a	6	$31\cdot7\pm0\cdot4$	$42\cdot6\pm0\cdot6$	$25\cdot7\pm0\cdot5$	$0\cdot53:0\cdot47$
	b/b	6	$34\cdot6\pm1\cdot1$	$47\cdot2\pm1\cdot2$	$18\cdot2\pm0\cdot8$	$0\cdot58:0\cdot42$
	b/c	6	$30\cdot7\pm0\cdot6$	$46\cdot8\pm0\cdot7$	$22\cdot5\pm0\cdot8$	$0\cdot54:0\cdot46$
Minor	b/a	6	$20\cdot6\pm0\cdot8$	$50\cdot5\pm0\cdot5$	$28\cdot9\pm0\cdot6$	$0\cdot46:0\cdot54$
	b/b	6	$23\cdot8\pm0\cdot7$	$47\cdot9\pm1\cdot1$	$28\cdot2\pm0\cdot8$	$0\cdot48:0\cdot52$
	b/c	6	$20\cdot3\pm0\cdot6$	$50\cdot2\pm0\cdot8$	$29\cdot5\pm0\cdot7$	$0\cdot45:0\cdot55$

* Minor allozymes were quantified from plates that were overstained for the major allozymes. Approximately 10 % of the GPI-1 activity was present in the minor set of allozymes. There was not always complete separation between GPI-1A (major) and GPI-1B (minor). See Fig. 2.

† a/b = (DBA × B)F$_1$ ♂♂; b/b (experiment 1) = (129 × B)F$_1$ ♂♂; b/a = (B × DBA)F$_1$ ♂♂; b/b (experiment 2) = (B-$Gpi\text{-}1s^a$ × B)F$_1$ ♂♂; b/c = (B × 101)F$_1$ ♂♂.

‡ Samples of spermatozoa were applied to the electrophoresis plate using a finely drawn-out Pasteur pipette in experiment 1 and a Helena Super Z applicator in experiment 2.

The allozyme ratios produced by the pancreas and mesentery samples showed no significant difference between the three genotypes but in the pancreas, in particular, the GPI-1B allozyme was consistently reduced. Whatever causes this reduction acts independently of the *Gpi-1t* genotype.

These results show that the new $Gpi\text{-}1t^c$ variant is not expressed in any of the somatic tissues tested. The question remains whether *Gpi-1t* is restricted in expression to female germ cells or is also expressed in the male germ line.

The ratios of GPI-1 allozymes produced by samples of epididymal spermatozoa from $Gpi\text{-}1s^a/Gpi\text{-}1s^b$ males of various *Gpi-1t* genotypes are shown in Table 7 and illustrated in Plate 1. Fig. 2. The spermatozoa samples produced a minor set of GPI-1 allozymes that migrated more slowly towards the cathode than the major set and comprised approximately 10 % of the total GPI-1 activity. These minor allozymes are more clearly separated from the major allozymes than the sub-bands that are sometimes produced by somatic tissue samples on cellulose acetate electrophoresis. Buehr & McLaren (1981) reported that similar minor GPI-1 allozymes were produced by samples of vas deferens spermatozoa. It is not yet clear whether both sets of allozymes are produced by the spermatozoa or whether one

set is produced by a somatic contamination of the sample. The experiments reported by Buehr & McLaren (1981) and experiments involving mixtures of blood and sperm samples (West & Fisher, unpublished) suggest that if only one set of allozymes is produced by the sperm it is the minor set.

In the experiments shown in Table 7 both sets of allozymes were quantified separately. Two electrophoresis plates were run for each set of samples. One plate was stained to quantify the major bands and the other was stained for longer, so that it was correctly stained for the minor allozymes but overstained for the major. The results show that the *Gpi-1t* genotype of the male has no effect on either the minor allozyme ratios or the major allozyme ratios. The GPI-1A monomer tended to be in slight excess in the major allozymes (as it was in the somatic tissues) but the GPI-1B monomer tended to predominate in the minor allozymes (as in the unfertilized eggs from *Gpi-1s^{a}t^b/Gpi-1s^{b}t^b* females). It is not clear whether this difference is significant since there was not always complete separation between the major GPI-1A allozyme and the minor GPI-1B allozyme. Whatever the origin of the major or minor allozymes it is clear that *Gpi-1t* is not expressed in either set.

4. DISCUSSION

The analysis of the effects of the new variant on GPI-1 expression in unfertilized eggs, somatic tissues and spermatozoa, together with the genetic linkage experiment, confirm that it is a variant of the *Gpi-1t* temporal locus. We designate this new allele *Gpi-1t^c*.

The concordance of the GPI-1 monomer ratios, in eggs from females heterozygous for both *Gpi-1s* and *Gpi-1t*, with the ratios of GPI-1 activity produced by eggs from the three *Gpi-1t* homozygotes (Tables 3 and 4; Peterson & Wong, 1978) implies that *Gpi-1t* is *cis*-acting and exerts its control before dimer formation. Moreover, the agreement between the observed allozyme ratios and those expected from the monomer ratios (Table 3) argues that monomers aggregate at random, so regulation of GPI-1 activity is achieved by synchronous expression of *Gpi-1s*. (Asynchronous gene expression in *Gpi-1s^a/Gpi-1s^b* heterozygotes would result in a period when only one type of monomer was produced and so the allozyme ratios would differ from those predicted by $p^2 : 2pq : q^2$.) From this it seems that *Gpi-1t* regulates GPI-1 activity either by controlling the rate of monomer synthesis from *Gpi-1s* or by controlling the rate of degradation of those monomers.

The experiments with other tissue samples show that the expression of *Gpi-1t* is restricted to female germ cells. It is particularly relevant that the temporal locus is not expressed either in spermatozoa or in the cumulus cells which, as granulosa cells, were in intimate contact with the maturing oocyte.

Green (1981, pp. 95–6) suggested designating haplotypes for the different combinations of *Gpi-1s* and *Gpi-1t* that are known. Five haplotypes of the *Gpi-1* gene complex can now be defined. (The *Gpi-1s^d* allele is excluded for now because it only exists in feral mice (Padua, Bulfield & Peters, 1978) and associated *Gpi-1t* genes are not yet known.) Suggested haplotype designations are shown in Table 8. Haplotypes [*Gpi-1*]^b and [*Gpi-1*]^d are designated as suggested by Green (1981)

but $[Gpi-1]^a$ is used in preference to $[Gpi-1]^{lp}$. (On the basis of enzyme activity measurements (Peterson & Wong, 1978) LP/J is probably $Gpi-1t^b$ but, in the absence of electrophoretic data, there is a possibility that LP/J may have a $Gpi-1t$ allele with an effect intermediate between $Gpi-1t^b$ and $Gpi-1t^c$.)

Table 8. *Suggested* Gpi-1 *haplotype designations*

Suggested haplotype	Gpi-1s	Gpi-1t	Strains	References
$[Gpi-1]^a$	a	b	LP	Peterson & Wong, (1978)
			A2G	McLaren & Buehr, (1981)
			129, B-$Gpi-1s^a$	West & Green, (1983)
			A*, BALB/c, IS	Present results
$[Gpi-1]^b$	b	b	B6*	Peterson & Wong, (1978)
			B	McLaren & Buehr, (1981)
			CBA, C3H, JU, JUN, JUW, SM†, TFH	Present results
$[Gpi-1]^c$	c	b?	B-$Gpi-1s^c$*	West & Green, (1983)
$[Gpi-1]^d$	a	a	DBA*	Peterson & Wong, (1978)
			MR	McLaren & Buehr, (1981)
			B10-p (and by inference NZW)	Present results
$[Gpi-1]^k$	a	c	101*, B-c (and by inference AKR)	Present results

* Identifies the suggested type strain. Where possible, haplotype symbols are abbreviations of the type strain. The haplotype $[Gpi-1]^k$ is the exception: the symbol 'k' represents AKR but this is not used as a type strain because it has not been tested directly.

† Not all stocks of SM are $Gpi-1s^b$ (see footnote to Table 1).

All nine of the $Gpi-1s^b$ strains so far examined carry the $Gpi-1t^b$ temporal allele (Table 8). However, strains B and B6 are closely related and JUN and JUW were derived from crosses between JU and CBA, so only six of these strains are independent. It remains to be seen whether $Gpi-1s^b$ exists, in other strains, in association with $Gpi-1t^a$ or $Gpi-1t^c$.

The discovery of $Gpi-1t^c$ shows that there are at least three alternative developmental programmes for determining levels of glucose phosphate isomerase in the mouse oocyte.

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