ELECTROPHORETIC VARIATION FOR *X*-CHROMOSOME-LINKED PHOSPHOGLYCERATE KINASE (PGK-1) IN THE MOUSE

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ABSTRACT

Electrophoretic variation for *X*-chromosome-linked phosphoglycerate kinase (PGK-1) has been found as a polymorphism in feral mice in Denmark. Males from feral sampling or from a variety of genetic crosses have only a single-banded phenotype of the variant PGK-1A type or of the PGK-1B type commonly found among inbred mice. By contrast, three phenotypes were observed among females; two homozygous single-banded types and a heterozygous double-banded type. The *X*-chromosome linkage of the *Pgk-1* locus was determined from the mode of inheritance in F_1 and backcross generations and confirmed by the linkage of *Pgk-1* and the *X*-linked markers *Hq*, *Ta* and *Mo*. *Pgk-1* showed 29/122 recombinations with *Hq*, 5/185 with *Ta* and 0/108 recombinants with *Mo*. Based on these recombination data, a gene order of *Hq—Ta—Pgk-1—Mo* is suggested.

THE *X*-chromosome linkage of the gene coding for phosphoglycerate kinase (PGK, E.C. 2.7.2.3.) has been determined in humans by means of an electrophorectic polymorphism (CHEN *et al.* 1971) and by an enzyme activity deficiency associated with hemolytic anemia (VALENTINE *et al.* 1969). The inheritance pattern of electrophoretic variants of PGK in marsupials also follows that expected for an *X*-chromosome gene (COOPER *et al.* 1971).

To date, no structural variants have been reported for the *X*-chromosome linked PGK in *Mus musculus*, either from surveys of inbred strains (OMENN and COHEN 1971; KOZAK, MCLEAN and EICHER 1974) or from feral mouse populations (CHAPMAN, unpublished). The *X*-chromosome linkage of PGK has been indirectly determined in the mouse by comparing the PGK activity levels in oocytes from *XX* and *XO* females (KOZAK, MCLEAN and EICHER 1974) and by somatic cell genetic analysis of single-cell derived clones (CHAPMAN and SHOWS 1976). In the latter study the clones were made from a primary culture of a female hybrid fetus obtained from an artificial insemination mating between the two Mus species, *Mus caroli* and *Mus musculus*. The electrophoretic mobility of PGK is different in these two species, and the clones expressed either the *M. caroli* form or the *M. musculus* form as predicted by the single-active-X-chromosome hypothesis (LYON 1961).

In this paper we report an electrophoretic variant for PGK in the mouse,

found as a polymorphism in a population of feral *Mus musculus musculus* in Denmark, and give direct evidence for the *X*-chromosome linkage of the *Pgk-1* locus in this species.

MATERIALS AND METHODS

In the autumn of 1975 a total of 186 mice were trapped in 16 different locations in Denmark in the regions of Jutland, Fynen, and Zealand. Polymorphism for PGK was observed in two localities about 30 km apart, west of Aarhus in central Jutland. Thirty-six animals were obtained from these two sites.

Males with the variant form of PGK were crossed to C3H females or to females carrying one of the following *X*-linked genes: tabby (*Ta*); brindled (Mo^{br}) or harlequin (*Hq*). The C3H strain is maintained as a separate inbred colony at the Genetics Institute, Aarhus University. The marker stocks used in this study were kindly provided by Dr. Anne McLaren, MRC Mammalian Development Unit. University College, London; Dr. Mary Lyon, Harwell; Mr. Jack Issacson, Department of Genetics, University of Edinburgh and Dr. Eva Eicher, The Jackson Laboratory, Bar Harbor. Hybrid female progeny were backcrossed to C3H or variant males and the relative position of the locus for PGK on the *X* chromosome was established by analyzing 415 offspring from mothers heterozygous for both PGK and one of the *X*-linked genes.

The electrophoretic phenotypes of PGK were determined from hemolysates or kidney homogenates. The samples were applied to filter paper wicks and inserted into horizontal starch gels (12% Electrostarch). Electrophoresis was carried out in a Tris-citrate system, pH 7.0 (0.225 M tris, 0.071 M citric acid for the bridge buffer and diluted 1:25 for the gel). PGK activity was observed on the gels using the staining technique of Beutler (1969).

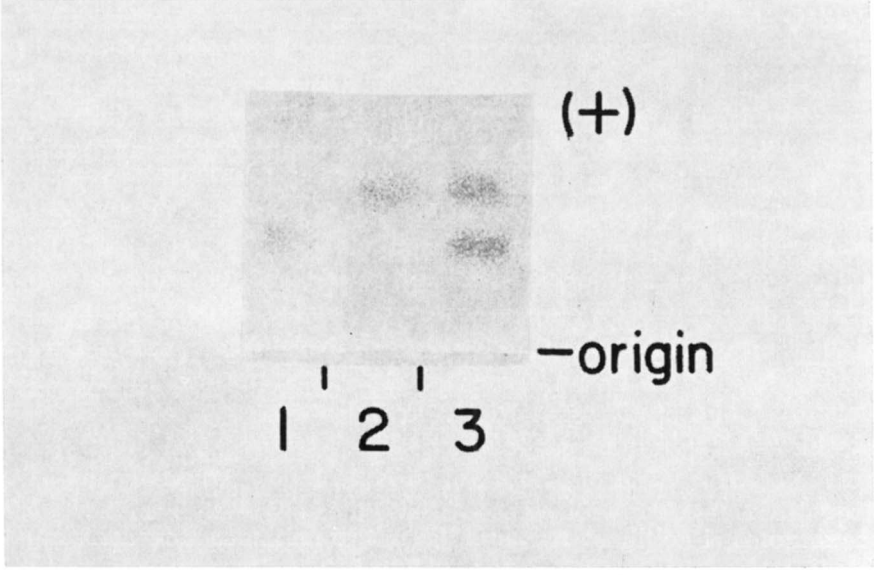

Figure 1.—Electrophoretic phenotype of PGK-1.
(1) PGK-1B: electrophoretic form common to inbred strains.
(2) PGK-1A: variant form found in Danish feral mice.
(3) PGK-1AB: heterozygous form seen only in females.

RESULTS

Electrophoretic phenotypes

Most of the mice sampled in Denmark had the same electrophoretic mobility for PGK as that observed for inbred strains of *Mus musculus*. This single-banded type (Figure 1;1) is designated PGK-1B. Among 11 males from the locality Bjertrup, 8 had the PGK-1B type, while 3 had a new single-banded, faster migrating type, designated PGK-1A (Figure 1;2). Of 19 females trapped in Bjertrup, 10 had the single slow band (PGK-1B), one had the single fast band (PGK-1A), and the remaining 8 had both bands (PGK-1AB) (Figure 1;3). The same electrophoretic form of PGK was also found in another trapping area, Tinning, about 30 km from Bjertrup. Only 6 mice were caught in Tinning and these were typed as follows: 1 PGK-1B male, 1 PGK-1A male, 2 PGK-1AB females and 2 PGK-1A females. The distribution patterns of the PGK-1 phenotypes in males (hemizygous expression) and females (heterozygous expression) suggest *X*-chromosome linkage of the PGK variation.

Segregation of PGK-1

The initial indication of *X*-chromosome linkage was confirmed by the matings given in Table 1. When PGK-1B females were crossed to PGK-1A males, the female progeny were uniformly PGK-1AB, while the male offspring had the same phenotype as the mother, PGK-1B. In backcross matings of PGK-1AB females with PGK-1A males, the male offspring were either PGK-1A or PGK-1B in a ratio of 46:37, respectively, while female progeny were either PGK-1A or PGK-1AB in a ratio of 31:40. The reciprocal backcross, PGK-1AB females × PGK-1B males, segregated 95 PGK-1A:79 PGK-1B males and 91 PGK-1AB: 106 PGK-1B females.

In both backcrosses the segregation of males into PGK-1A and PGK-1B does not significantly differ from a 1:1 ratio ($\chi^2 = 2.431$, $p = 0.15$). For females the ratio of homozygous PGK-1A or PGK-1B to heterozygous PGK-1AB in both backcrosses is also consistent with a 1:1 segregation ratio ($\chi^2 = 0.134$, $p = 0.70$). These results clearly demonstrate that the gene determining the electrophoretic variant for PGK-1 segregates as an *X*-linked locus. We designate this locus *Pgk-1*,

TABLE 1

Segregation of PGK-1 among progeny of F_1 and backcross matings

Parents ♀	Parents ♂	Phenotype of progeny PGK-1 ♀	PGK-1 ♂	PGK-1AB ♀	PGK-1AB ♂	PGK-1B ♀	PGK-1B ♂	Total
Phenotype								
PGK-1B	PGK-1A	..	..	11	..	..	12	23
PGK-1AB	PGK-1A	31	46	40	..	..	37	154
PGK-1AB	PGK-1B	..	95	91	..	106	79	371
Total		31	141	142	..	106	128	548

with *Pgk-1*^{a} as the allele responsible for the PGK-1A variant and *Pgk-1*^{b} as the allele responsible for the PGK-1B variant.

Linkage of Pgk-1

The position of *Pgk-1* on the *X* chromosome was determined from a series of crosses with the sex-linked genes harlequin (*Hq*), tabby (*Ta*) and brindled (*Mo*^{br}).

In linkage tests of *Pgk-1* and *Hq*, heterozygous females with the genotype *Hq Pgk-1*^{b}/+ *Pgk-1*^{a} were mated with either + *Pgk-1*^{a}/*Y* males (Table 2; cross A) or with + *Pgk-1*^{b}/*Y* males (Table 2; cross B). We observed 14 recombinants among 50 offspring in cross A and 15/72 in cross B. From these data, the estimated recombination for *Hq* to *Pgk-1* is 23.8 ± 3.9%.

In crosses with *Ta* heterozygous females, (*Ta Pgk-1*^{b}/+ *Pgk-1*^{a}) were mated with + *Pgk-1*^{a}/*Y* or + *Pgk-1*^{b}/*Y* males (Table 3; cross A and B, respectively). Among 185 offspring 5 recombinants were found and the estimate of *Ta* to *Pgk-1* recombination was 5/185, or 2.7 ± 1.2%.

In the third cross *Mo*^{br} *Pgk-1*^{b}/± *Pgk-1*^{a} females were mated with *Pgk-1*^{b} +/*Y* males (Table 4). Brindled male offspring do not typically surive post weaning; consequently, their PGK-1 type was determined in kidneys at approximately 14 days of age. No recombinants were observed among the 108 progeny tested. The confidence interval for the *Pgk-1* to *Mo* distance can be estimated from the formula $0 \leq r \leq 1-\alpha^{1/n}$, where r is the probability of recombination, n is the number of progeny tested and α is the level of significance. With $n = 108$ and $\alpha = 0.05$, *Pgk-1* is less than 2.8 units from *Mo* at the 95% confidence interval.

In a three-point cross involving the *Hq*, *Ta* and *Mo*^{br} loci, ISAACSON, STEWART and FALCONER (1974) reported a map order of *Hq–Ta–Mo*^{br} with the recombina-

TABLE 2

Cross I: Recombination between Hq *and* Pgk-1

A. *Hq Pgk-1*^{b}/ + *Pgk-1*^{a} × + *Pgk-1*^{a}/ *Y*

Nonrecombinant	*N*	Recombinant	*N*
+ *Pgk-1*^{a}/ *Y*	18	+ *Pgk-1*^{b}/ *Y*	6
Hq Pgk-1^b/ *Y*	8	*Hq Pgk-1*^{a}/ *Y*	1
+ *Pgk-1*^{a}/ + *Pgk-1*^{a}	13	+ *Pgk-1*^{b}/ + *Pgk-1*^{a}	4
Hq Pgk-1^b/ + *Pgk-1*^{a}	18	*Hq Pgk-1*^{a}/ + *Pgk-1*^{a}	4
Total	57		15

B. *Hq Pgk-1*^{b}/ + *Pgk-1*^{a} × + *Pgk-1*^{b}/ *Y*

Nonrecombinant	*N*	Recombinant	*N*
+ *Pgk-1*^{a}/ *Y*	9	+*Pgk-1*^{b}/ *Y*	2
Hq Pgk-1^b/ *Y*	3	*Hq Pgk-1*^{a}/ *Y*	7
+ *Pgk-1*^{a}/ + *Pgk-1*^{b}	11	+ *Pgk-1*^{b}/ + *Pgk-1*^{b}	3
Hq Pgk-1^b/ + *Pgk-1*^{b}	13	*Hq Pgk-1*^{a}/ + *Pgk-1*^{b}	2
Total	36		14

Combined % recombination ± S.E. = 29/122 = 23.8 ± 3.9%

TABLE 3

Cross 2: Recombinations between Ta *and* Pgk-1

A. *Ta Pgk-1^b/ + Pgk-1^a × + Pgk-1^a/ Y*

Nonrecombinant	*N*	Recombinant	*N*
+ Pgk-1^a/ Y	20		
Ta Pgk-1^b/ Y	14	*Ta Pgk-1^a/ Y*	1
+ Pgk-1^a/ + Pgk-1^a	11	*+ Pgk-1^b/ + Pgk-1^a*	1
+ Pgk-1^a/ Ta Pgk-1^b	10		
Total	55		2

B. *Ta Pgk-1^b/ + Pgk-1^a × + Pgk-1^b/ Y*

Nonrecombinant	*N*	Recombinant	*N*
+ Pgk-1^a/ Y	30	*+ Pgk-1^b/ Y*	1
Ta Pgk-1^b/ Y	33	*Ta Pgk-1^a/ Y*	1
+ Pgk-1^a/ + Pgk-1^b	24	*+ Pgk-1^b/ + Pgk-1^b*	1
Ta Pgk-1^b/ + Pgk-1^b	38		
Total	125		3

Combined % recombination ± S.E. = 5/185 = 2.7 ± 1.2%

tion estimates of 20.5 ± 2.1% between *Hq* and *Ta* and 5.5 ± 1.2% between *Ta* and *Mo^{br}*. They found 22 recombinants between *Ta* and *Mo^{br}* (390 progeny). By a contingency table χ^2 test, this distance is not significantly different from the *Ta–Pgk-1* recombination distance of 5/185, or 2.7 ± 1.2%, that we observed ($\chi_1^2 = 2.42$; $p = 0.1$). The specific map order of *Pgk-1* and *Mo^{br}* related to *Ta* cannot be deducted from these data and will require a three-point linkage test. By comparing the distance of *Hq–Pgk-1*, *Ta–Pgk-1* and *Pgk-1–Mo^{br}* with the findings of ISAACSON, STEWART and FALCONER (1974), we estimate that the most likely gene order is *Hq–Ta–Pgk-1–Mo^{br}*.

DISCUSSION

The relative activities of the two electrophoretic forms of PGK-1 are similar

TABLE 4

Cross 3: Recombination between Pgk-1 *and* Mo^{br}

Pgk-1^b Mo^{br}/ Pgk-1^a + × Pgk-1^b +/Y

	N
Pgk-1^a +/Y	28
Pgk-1^b Mo^{br}/Y	18*
Pgk-1^a +/+ Pgk-1^b	28
Pgk-1^b Mo^{br}/+ Pgk-1^b	34
Total	108

% Recombination = 0/108

* *Mo^{br}/Y* males were killed at 14 days and *Pgk-1* determined in kidney homogenates.

in the qualitative gel staining procedure. That is, the PGK-1A band has the same staining intensity as the PGK-1B band when equal amounts of PGK-1A and PGK-1B homogenates are mixed. These findings suggest that there is no gross alteration in enzyme activity associated with either electrophoretic form. Similar findings were reported by CHEN *et al.* (1971) for the electrophoretic variation of PGK in humans. However, in view of the importance of PGK function in red cells and the observation that a deficiency for PGK in humans is associated with hemolytic anemia (VALENTINE *et al.* 1969), a more complete characterization of the properties of the PGK-1A variant may be useful for determining whether a physiologically significant difference is associated with this allelic form. Such a physiological difference between PGK-1 allelic forms may be important for assessing selective pressures for PGK-1 variation as well as evaluating *X*-chromosome expression in females.

In our sampling of mice, we observed polymorphism for PGK-1 in two sites about 30 km apart in Central Jutland. The variant form from both locations, Tinning and Bjertrup, has the same electrophoretic mobility and was common in samples from both locations. From Tinning, 7 of 10 chromosomes carried the *Pgk-1*^{a} allele. From Bjertrup, where 11 males and 19 females were trapped, the frequency of the *Pgk-1*^{a} allele was $P = 0.27$ among males and $P = 0.26$ among females. Although the frequency estimates are based upon a small number of animals, the similarity of the two estimates between sexes suggests that the *Pgk-1*^{a} variant is well established in the Bjertrup population. Attempts to find additional variant mice near or between the two locations were not successful, suggesting that the distribution of the *Pgk-1*^{a} allele is fairly limited in Denmark. An assessment of the distribution of the variant allele would require a more thorough and systematic sampling.

Among the heterozygous females from wild trapped mice and those from various crosses, the relative intensities of PGK-1A and PGK-1B bands is variable. The findings are predicted by the LYON or "single-active-*X*-chromosome" hypothesis in which females are mosaic with a single *X* chromosome functionally inactive in every cell (LYON 1961). We have not quantitatively determined the ratios of PGK-1A and PGK-1B activity in heterozygous females. If it should ultimately prove that either of the PGK-1 forms were predominately expressed in heterozygotes, it will be necessary to separate the effects of nonrandom *X*-inactivation caused by *X*-chromosome controlling elements, such as the *Xce* locus (CATTANACH and ISAACSON 1967), from the possible effects of embryonic selection for cells expressing one of the *Pgk-1* allelic forms.

The occurrence of polymorphism for *Pgk-1* in feral mice further demonstrates the importance and the utility of surveying the feral *Mus musculus* gene pool to find variation not present among inbred strains. In this instance the PGK-1 variation found in Denmark represents the first electrophoretic variant for an *X*-chromosome linked enzyme in the mouse. As such it should prove valuable as a tool for studying a number of features of *X*-chromosome dosage compensation in an experimental mammalian system.

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