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The γ phosphorylase kinase gene, *Phkg*, maps to mouse Chromosome 5 near *Gus*

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Abstract. Phosphorylase kinase is a multimeric regulatory enzyme in the glycogenolytic pathway. Interest in various types of phosphorylase kinase enzyme deficiency has focused attention on cloning and mapping the enzyme subunits. We report the mapping of the catalytic γ subunit gene, *Phkg,* to mouse Chromosome (Chr) 5 near β-glucuronidase (*Gus*), between alpha fetoprotein (*Afp*) and erythropoietin (*Epo*). In addition, PCR-based polymorphism assays have been developed for the human (EPO) and mouse erythropoietin genes, and a unique recombinant inbred strain distribution pattern has been defined for *Epo,* a distal anchor marker on mouse Chr 5.

Introduction

Phosphorylase kinase (Phk) is an intermediate enzyme in the glycogenolytic regulatory cascade. This multimeric enzyme is activated by phosphorylation via the β-adrenergic pathway and by calcium ions released by neural stimulation. Activated Phk phosphorylates the enzyme glycogen phosphorylase, which releases glucose-6-phosphate from glycogen (reviewed by Picket-Gies and Walsh 1986). Tissue-specific isoforms of each of the Phk subunits have been described and presumably regulate the activity of the holoenzyme in various tissues. The regulatory subunits, α and β, have been cloned from rabbit and mapped in human and mouse (Zander et al. 1988; Francke et al. 1989; Barnard et al. 1990; Davidson et al. 1992; Harmann et al. 1991). A family of calmodulin genes to which the δ subunit belongs has also been cloned (Nojima 1989).

The gene encoding the skeletal muscle isoform of γPhk (*Phkg*) has been cloned from mouse and rat (Maichele et al. 1993; Cawley et al. 1993). In the current study we have identified polymorphisms in *Phkg* and have used those markers to map the gene to mouse Chr 5 near β-glucuronidase (*Gus*). A new polymorphism in the mouse erythropoietin (*Epo*) gene, a distal Chr 5 reference marker, was also developed for use in mapping with the recombinant inbred strain AKXL and with interspecific backcrosses. The linkage of *Phkg* to *Gus* and *Epo* in the mouse suggests human Chr 7q21-q22, which contains the human genes GUSB and EPO, as the most likely location for the human homolog, PHKG.

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Materials and methods

Construction of the intersubspecific backcross (DF/B-*df/df* × CASA/Rk) × DF/B-*df/df,* and isolation of DNA from these animals has been described previously (Buckwalter et al. 1991; Lossie et al. 1993). DNA from *Mus musculus domesticus* (strain WSB) was a gift from P. Tucker (University of Michigan). DNA from CASA/Rk, MOLD/Rk, *Mus musculus musculus, Mus musculus domesticus poschiavinus,* and the recombinant inbred strain AKXL was obtained from The Jackson Laboratory.

Polymerase chain reactions (PCR) were performed as previously described with one radiolabeled primer in each reaction (Maichele and Chamberlain 1992). Reaction products were analyzed on 6% denaturing polyacrylamide gels, followed by autoradiography. Primer sequences for *Afp* (Aitman et al. 1991), *Gus* (Hearne et al. 1991), and the *Phkg* B2 repeat insertion (Maichele et al. 1993) have been published. *D5Mit27* oligonucleotide primers were purchased from Research Genetics and used at an annealing temperature of 55°C (Dietrich et al. 1992a, 1992b). Additional forward and reverse primers and annealing temperatures are as follows:

*Phkg*4A: 61°C; F: GAC AAC AGT TGA TTC AGG GCC; R: TAA ACA ACC TCC CCC ACC C
*Phkg*4B: 61°C; F: GAC ATG AAC TAC CAC CAG CAG C; R: GCT GGG ACT AAA GGT ATG GGC
*Phkg*4C: 60°C; F: CTG TGA GAT TCA GAC CAG CCT G; R: GGG CTG AAT TAA AGG CAT GC
Epo: 59°C; F: GTG TGG GAG AAA ATA TCA GAG ACA; R: AAT GTC ATT CCC TAT CCT CCC T

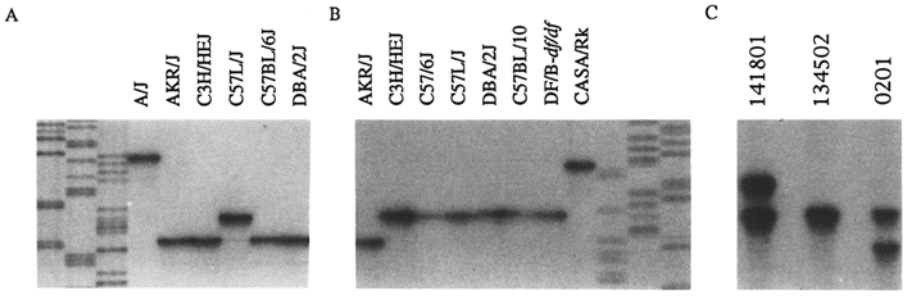

Fig. 1. The range of allele sizes in some of the RI parental strains at *Phkg4A* (A) and *Epo* (B). Allele sizes were determined by reference to previously sequenced alleles and a one-base-pair ladder generated from pTZ19R (see Table 1). (C) The three EPO alleles in the CEPH parents.

Table 1. Strain genotypes at polymorphic loci within *Phkg* and *Epo*. Simple sequence repeats and B2 polymorphisms are described in the text. PCR products were amplified from genomic DNA from eight strains and seven species of mice and resolved on denaturing polyacrylamide gels. Sequencing ladders from pTZ19R were used in conjunction with PCR products from previously sequenced alleles as molecular size standards. AKR/J and C57L/J are the progenitors of recombinant inbred strain AKXL. DF/B-*df/df* and CASA/Rk are the backcross parental strains.

	BALB/c	A/J	DF/B-*df/df*	AKR/J	C3H/HEJ	DBA/2J	C57BL/6J	C57BL/10	C57L/J	*M.m.dom.* WSB	*M.m.dom. posch.*	MOLD/Rk	CASA/Rk	*M. musculus*	*M. spretus*
Phkg4A	150	150	150	147	147	147	147	147	147	148	165	137	[a]	147	230–250 variable[b]
Phkg4B	105	105	105	108	108	108	108	108	108	108	104	106	93	99	103,116[b]
Phkg4C	242	242	242	222	222	222	222	222	227	232	232	272	177	297	97,240,254,340,350
Phkg exon 10 B2	+	+	+	−	−	−	−	−	−	−	+	+	+	−	−
Epo intron 3	163	163	163	159	163	163	163	163	163	[c]	[c]	171	171	[c]	[a]

[a] Did not amplify.
[b] Not all samples amplify.
[c] Not tested.

EPO: 59°C; F: AAT GAG GGC TGT ATG GAA TAC A; R: AGC TGA GCA AAC AGA AGG TAT G

Results and discussion

Polymorphic sequences in Phkg

While characterizing the structure of *Phkg*, we observed several potentially polymorphic short tandem repeat (STR) sequences within intron 4 (Weber and May 1989; Hearne et al. 1991). These STRs had the sequences $(AT)_{12}TC(T)_{25}$, $(T)_5AT(A)_{28}$, and $(AAAGG)_{13}$, and were designated *Phkg4A*, *Phkg4B*, and *Phkg4C*, respectively. Gel analysis of PCR products amplified from genomic DNA of various strains of mice demonstrated that each of these repeats is polymorphic (Table 1, Fig. 1). The seven analyzed inbred strains exhibited three *Phkg* haplotypes.

In addition to the STRs observed in intron 4, we have identified a B2 repeat present in *Phkg* exon 10 of certain strains and species of mice. The presence or absence of this repeat can be assayed by PCR and is associated with differences in the relative sizes and amounts of the two transcripts produced from the gene (Table 1; Maichele et al. 1993). *M. m. castaneus* (CASA/Rk) and *M. m. molossinus* (MOLD/Rk) contain the insertion, whereas *M. spretus* does not. Of particular interest is the fact that strains of *M. m. domesticus* (WSB and *poschiavinus*) collected in various locations differ in the presence or absence of this repeat insertion (Table 1).

Polymorphic sequences in Epo and EPO

Epo recently replaced *Zp-3* as the most distal Chr 5 backbone marker, based in part on the assumption that an STR was available for this locus (Kozak and Stephenson 1992). However, the original reference (Abbot 1992) described PCR primers that amplify a non-polymorphic sequence in the mouse *Epo* gene and that were used to screen mouse–hamster somatic cell hybrid panels for the presence of mouse Chr 5. While searching for potential polymorphisms, we observed an STR of sequence $(TTCA)_8$ within intron 3 of the published *Epo* sequence (McDonald et al. 1986). PCR analysis showed that this STR would be informative in crosses between AKR/J and the other tested laboratory strains, as well as in intersubspecific crosses with CASA/Rk or MOLD/Rk (Table 1 and Fig. 1). This *Epo* STR also defines a unique strain distribution pattern (SDP) for distal Chr 5 in the recombinant inbred strain AKXL (Table 2, Fig. 2).

The human erythropoietin gene sequence (Lin et al. 1985) also contains an imperfect STR in intron 3, in this case following an Alu repeat. When examined by single-strand conformation polymorphism (SSCP), this Alu repeat exhibited two alleles with a polymorphic information content (PIC value) of 0.06 (Orita et al. 1990; Botstein et al. 1980). To determine whether a more informative or easier to analyze polymorphism could be obtained by focusing directly on the STR, we used PCR primers flanking this region to amplify genomic DNA from 80 CEPH parents. Three alleles were detected (Fig. 1); the most common probably corresponds to the previously sequenced 108-bp allele (Lin et al. 1985; Orita et al. 1990) and has a fre-

Table 2. Strain distribution pattern of distal mouse Chr 5 loci typed in the AKXL RI strains. At each locus, alleles derived from AKR/J and C57L/J are represented by the letters A and L, respectively.

	AKXL strains																		
Locus	5	6	7	8	9	12	13	14	16	17	19	21	24	25	28	29	37	38	References
Pmv-11	L	L	L	A	L	A	L	A	L	A	A	L	L	L	L	L	L	A	Frankel et al. 1989
Bcd-1	A	A	L	L	L	A	A	A	A	L	L	L	L	A	L	L	L	L	Winchester et al. 1987
Fla	L	A	L	A	A	L	A	A	A	A	L	L	L	L	L	L	L	L	Winchester et al. 1987
Gus, D5Mit27, Phkg	L	A	L	A	A	L	A	A	A	A	L	L	L	L	L	L	L	L	Winchester et al. 1987; this work
Epo	A	A	L	A	A	L	A	A	A	A	L	A	A	L	L	L	A	A	This work

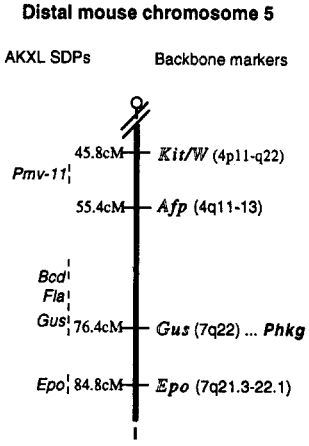

Fig. 2. Linkage map of distal mouse Chr 5 (after Kozak and Stephenson 1992). Backbone markers are shown in **open typeface** with the locations of their human homologs in **parentheses** and distances from the centromere to the left. The approximate locations of AKXL strain distribution patterns are shown in **dotted lines.** Human chromosomal locations are from: *Kit,* Hsieh et al. 1991; *Afp,* Theune et al. 1991; *Gus,* Allanson et al. 1988; *Epo,* Watkins et al. 1986; and Human Gene Mapping 11, 1991.

quency of 0.89. A second allele is four base pairs longer and occurs with a frequency of 0.106. CEPH DNA # 141801 is heterozygous for those two alleles. A third, rare allele of 104 bp was seen in one sample, CEPH DNA # 0201, corresponding to a frequency of 0.006. The PIC value of the EPO STR is 0.1824 in the CEPH parents, higher than that detected by SSCP in an unidentified population.

Mapping Phkg

Phkg and *D5Mit27* were mapped in the recombinant inbred strain AKXL. This strain was derived by crossing AKR/J and C57L/J and is therefore informative at *Phkg4C* (Taylor 1989; Table 1; Fig. 1). The SDP of *Phkg* in AKXL matched that of *Fla* and *Gus,* as did the SDP of *D5Mit27,* an STR which has recently been localized to *Gus* (Table 2; Winchester et al. 1987; Dietrich et al. 1992b). *Fla* and *Gus* previously defined the most distal SDP available on Chr 5 in AKXL; thus the candidate region for *Phkg* extended from *Bcd-1* to the telomere. Our determination of the SDP for *Epo* in AKXL excluded the most distal portion of Chr 5 (Table 2, Fig. 1).

To confirm this assignment, we mapped *Phkg* in an intersubspecific backcross. Eighty-nine backcross animals were typed for five STRs on distal Chr 5. Nineteen crossovers were detected between *Afp* and *Gus,* four between *Gus* and *Epo,* and none between *Gus, D5Mit27,* and

Table 3. Segregation of Chr 5 loci in the backcross (DF/B-*df/df* × CASA/Rk) × DF/B-*df/df.* Each column represents a chromosome inherited from an F_1 (DF/B-*df/df* × CASA/Rk) individual. Closed and open boxes represent DF/B and CASA/Rk alleles, respectively. The number of backcross animals that inherited each chromosome is indicated below.

	Chromosomes					
Afp	□	■	■	□	■	□
Gus, D5Mit27, Phkg	□	■	□	■	■	□
Epo	□	■	□	■	□	■
Total animals: 89	37	29	12	7	2	2

Phkg. No double recombinants were observed (Table 3). We estimate the distance between *Afp* and *Gus* to be 21.3 ± 4.3 cM. The 95% confidence interval is 11.3–31.3 cM, consistent with the published value of 19.7 ± 2.8 cM (Rothman and Ericson 1987; Geissler et al. 1988). The distance between *Gus* and *Epo* was estimated to be 4.5 ± 2.2 cM with a 95% confidence interval of 0–14.3 cM, in agreement with the previously reported values of 8.4 ± 2.0 cM (Singh et al. 1991) and 5.6 ± 2.2 cM (Rosnet et al. 1993).

The human homolog of *Phkg,* PHKG, has not been cloned, and polymorphisms are unavailable. PHKG has been mapped to human Chr 7 by Southern analysis of human–rodent somatic cell hybrid mapping panels with mouse or rabbit cDNA clones as probes (Chamberlain et al. 1987; Jones et al. 1990); both groups also observed cross-hybridizing sequences on Chrs 7 and 11. In situ hybridization with a rabbit γPhk cDNA clone produced a strongly hybridizing signal at human Chr 7p11-p12 and weaker signals at 7q21 and 11p11-p14 (Jones et al. 1990). The authors concluded that PHKG is probably located near the centromere of Chr 7; however, a cDNA probe might be expected to hybridize more strongly with a pseudogene than with the functional intron-containing gene. Linkage of *Gus, Phkg,* and *Epo* on distal mouse Chr 5 and the locations of GUSB and EPO on human 7q21-q22 (McAlpine et al. 1991) suggest 7q21-q22 as the likely location of PHKG.

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