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Genetics

Phosphoglucomutase 1: Complete human and rabbit mRNA sequences and direct mapping of this highly polymorphic marker on human chromosome 1

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ABSTRACT **A cDNA clone encoding the mRNA for the highly polymorphic human enzyme phosphoglucomutase 1 (PGM1; EC 5.4.2.2) has been isolated and characterized. This was achieved indirectly by first isolating a rabbit cDNA from an expression library using anti-rabbit PGM antibodies. A comparison of the nucleotide sequences shows that the homologies between human and rabbit PGM1 mRNAs are 92% and 97% for the coding nucleotide sequence and the amino acid sequence, respectively. The derived rabbit amino acid sequence is in complete agreement with the published protein sequence for rabbit muscle PGM. A physical localization of the human *PGM1* gene to chromosome 1p31 has been determined by *in situ* hybridization. Analysis of DNA from a wide variety of vertebrates indicates a high level of *PGM1* sequence conservation during evolution.**

Phosphoglucomutase (PGM; EC 5.4.2.2) catalyzes the interconversion of glucose 1-phosphate and glucose 6-phosphate and thus has a pivotal role in glycolysis and gluconeogenesis. The human enzyme is encoded by three autosomal unlinked structural loci, designated *PGM1*, *PGM2*, and *PGM3* (1, 2), and there is evidence for a fourth locus expressed in human milk (3). On the basis of protein studies, it is assumed that the loci arose by gene duplication and evolved separately to provide the present day isozymes, showing distinctive properties, such as molecular size (4), thermostability (5), substrate specificity (6), tissue distribution (7), and sulfhydryl group reactivity (8). Multiple isozymes, attributable to multiple *PGM* loci have been identified in a very wide range of species and it is evident that the initial *PGM* gene duplication(s) occurred in the remote evolutionary past.

Isozyme polymorphism of human *PGM1* was identified more than 25 years ago (9) and the locus has since been shown to exhibit a very high incidence of both common and rare allelic variation in all populations (10). The *PGM1* locus is an important anchor point for linkage analysis and for positioning markers on human chromosome 1p and its homologues in other species. The heterozygosity demonstrable by isozyme analysis of human erythrocytes [0.81 in the Centre d'Etude Polymorphisme Humain (CEPH) family panel for example] is comparable to several of the variable number tandem repeat loci and exceeds most of the restriction fragment length polymorphisms recognized by other probes on chromosome 1. The PGM1 isozyme polymorphism is a particularly useful marker of individuality in forensic science (11, 12) since the discriminating power of the *PGM1* locus (1 − probability of a match) is about 0.75 for the common isozyme phenotypes revealed by isoelectric focusing (12). Furthermore, the isozymes are found in a variety of body fluids and enzyme activity is retained for prolonged periods in stains recovered from scenes of crimes.

The extensive heterozygosity of *PGM1* and certain features of the variant isozyme patterns have led to speculation about the role of intragenic recombination in generating this diversity. The isozymes associated with the two alleles, *PGM1*1* and *PGM1*2*, discovered by starch gel electrophoresis, can be subdivided by isoelectric focusing into two classes: one designated "+" is more anodal than the other, designated "−". Thus four alleles ($PGM1*1^+$, 1^-, 2^+, and 2^-) define 10 common phenotypes (11). Furthermore, the properties of these common alleles 1 vs. 2 and + vs. − do not occur in a random association. Similarly, the isozymes encoded by the less-common PGM alleles *PGM1*3* and *PGM1*7* can also be subdivided by isoelectric focusing into + and − categories and here also the association of the latter property with the electromorphs (PGM1*3 vs. PGM1*7) is not random (13). These findings have led to the suggestion that much of the PGM1 diversity might be attributed to intragenic recombination. A gene phylogeny has been constructed for the eight most common alleles, in which intragenic recombination plays at least as significant a role as nucleotide substitution in the generation of allelic diversity (13).

In the present communication, we describe the isolation and the full sequence of both human and rabbit PGM1 mRNA. Antibodies raised in a sheep against purified rabbit muscle PGM cross react with human PGM1 (14) but are not sufficiently sensitive to detect human PGM1 expressed in phage expression vectors. Thus we approached the cloning of human PGM1 by using the antibodies to isolate a rabbit PGM1 cDNA clone, which was then employed as a human library screening probe. The cloning of the human PGM1 cDNA† will allow the hypothesis of the origin of the allelic diversity to be fully tested.

MATERIALS AND METHODS

Cloning of Human and Rabbit *PGM1*. Poly(A)$^+$ RNA was isolated from rabbit hind leg muscle by selective LiCl precipitation and binding to oligo(dT)-cellulose. A cDNA library (2×10^6 recombinants) was produced in the expression vector λgt11 using standard procedures with *Eco*RI adapters (15) and screened using antibodies raised in a sheep and specific for rabbit muscle PGM1 (14). Positive plaques were recloned three times to yield plaque-purified phage. cDNA inserts were removed by *Eco*RI digestion, gel-purified, and used to screen a human muscle cDNA library (15). The human PGM1 recombinants were similarly plaque-purified.

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Abbreviations: PGM, phosphoglucomutase; CEPH, Centre d'Etude Polymorphisme Humain.

†The sequence reported in this paper has been deposited in the GenBank data base (accession no. M83088).

Large-scale preparations of purified λ recombinants were carried out using a standard cesium chloride centrifugation procedure (16).

Subcloning and Labeling of cDNA Probes. Both human and rabbit cDNA inserts were subcloned into *Eco*RI sites of pUC18 and M13mp18 vectors. Preparations of plasmid or the replicative form of M13 were purified by equilibrium centrifugation in cesium chloride (16). cDNA inserts from pUC18 recombinants were gel-purified and labeled with ^{32}P using the oligonucleotide primer method (17).

DNA Sequence Analysis. M13mp18 subclones containing PGM1 cDNA inserts in opposite orientations were identified by restriction site mapping. Both cDNAs were sequenced by the dideoxynucleotide chain-termination sequencing method of Sanger *et al.* (18). For rabbit PGM1, intact cDNA and various fragments obtained by restriction enzyme digestion with *Kpn* I, *Hin*dIII, or *Sph* I were sequenced in both directions and, for the human PGM1, several *Hin*fI fragments were sequenced in both directions and a series of overlapping bidirectional deletions using exonuclease III (19) were created and sequenced.

Nucleic Acid Gel Blot Analysis. For Southern blots, genomic DNA was extracted, digested, electrophoresed on 0.8% agarose, and transferred to Hybond N^+ (Amersham). Total and poly(A)$^+$ RNA was purified from cell cultures or tissue samples fractionated by 3-(*N*-morpholino)propanesulfonic acid (Mops)/formaldehyde electrophoresis (16) and blotted onto GeneScreen membrane (NEN). Insert cDNA (25 ng) was labeled using [^{32}P]dCTP to a specific activity of 1×10^8 cpm/μg for both Northern and Southern blots. Southern blots were hybridized in 10% (wt/vol) dextran sulfate/5× standard saline citrate (SSC)/0.1% SDS at 65°C and Northern blots were hybridized in 50% (vol/vol) formamide/5× SSPE (1× SSPE = 0.18 M NaCl/10 mM sodium phosphate, pH 7.4/1 mM EDTA) at 42°C. Membranes were washed in 1× SSC/0.1% SDS at 65°C for Southern blots and in 0.2× SSC/0.1% SDS at 50°C for RNA analysis. Autoradiography was at −70°C.

***In Situ* Hybridization.** *In situ* hybridization was carried out on spreads of human metaphase chromosomes by using a biotinylated whole PGM1 cosmid as probe as described (20) but with certain modifications. Human Cot_1 DNA, a fraction of total DNA in which highly repetitive sequences are prevalent, was used at a 50-fold excess of probe concentration to suppress hybridization to these sequences. Signal detection was achieved using fluorescein isothiocyanate-conjugated avidin and amplified by alternate applications of biotinylated anti-avidin and fluorescein isothiocyanate-conjugated avidin. Preparations were mounted in anti-fade solution *p*-phenylenediamine dihydrochloride to which the fluorochromes diamidinophenylindole and propidium iodide had been added for counterstaining and banding. Preparations were evaluated using epifluorescence microscopy. Separate images were obtained for each fluorochrome, collected electronically by laser scanning (Bio-Rad MRC 600 confocal microscope), and merged. Signal position was compared with R-banding patterns for at least 10 metaphases showing signal on both chromatids with negligible background.

PCR Analysis of Somatic Cell Hybrids. Details of the somatic cell hybrids used are listed either in Carritt *et al.* (21) or Wong *et al.* (22). Hybrid DNA or human or rodent parent DNA (3 μg) was amplified using 25-mer oligonucleotides from the 3′ untranslated region of human *PGM1* (see Fig. 1) as primers. After 30 cycles of amplification, each consisting of 15 s at 94°C, 30 s at 58°C, and 30 s at 72°C, 10 μl was removed and analyzed on a 2.0% agarose gel. Each hybrid DNA was also amplified with primers to rodent Trp53 pseudogene (C. Abbott, personal communication) as a control to establish that all samples were amplifiable.

RESULTS

cDNA Clones for Human *PGM1*. Two human *PGM1* recombinants, λHPGM1.1 and λHPGM1.2, appeared to contain full-length cDNA inserts and detected an mRNA of ≈2.3 kilobases after Northern blot analysis of human skeletal muscle RNA. The length of the human cDNA inserts was 2320 base pairs (bp) and the length of the rabbit insert (from clone λRPGM) was 2279 bp. Each contained an open reading frame of 1686 bp; when translated there was 97% homology between the human and rabbit derived amino acid sequences (Fig. 1) and complete agreement between the derived rabbit amino acid sequence and the published rabbit protein sequence for muscle PGM1 (23). The positions of the 18 amino acid substitutions found between the human and rabbit PGM1 are shown diagrammatically in Fig. 2. A region of 83 amino acids spanning the active site is completely conserved, with serine at position 116. Ser-116 is known to react in a phosphorylated form directly with the substrate glucose 1-phosphate (24). There is complete conservation between residues 220 and 417 except for a single amino acid difference at position 345 where serine is present in the human protein and asparagine is in the rabbit. In contrast, the COOH-terminal region is less well conserved with nine substitutions within the final 145 residues. The amino acid differences between the species account for a relatively high positive charge on the rabbit protein, which is reflected in the more basic isoelectric point (14). The molecular weights derived from the rabbit and human amino acid sequences are 61,600 (23) and 61,300, respectively; the latter is somewhat larger than the crude estimate of 51,000 obtained by gel-filtration chromatography of human placental PGM1 (4).

The cDNAs include a 5′ untranslated region of 62 bp and 39 bp and a 3′ untranslated region of 572 bp and 554 bp in the human and rabbit clones, respectively. The overall homology between the human and rabbit coding sequences is 92%. There is a moderate degree of homology at the 5′ untranslated region which is G+C-rich (G+C = 76% and 80%, respectively) in both species; without sequence analysis or methylation studies of the 5′ flanking region, it is not certain whether this represents a Hpa II tiny fragments (HTF) island, but such a feature might be expected in a ubiquitously expressed gene like *PGM1*. The sequence homology between the 3′ untranslated regions is generally less but there are several segments extending over at least 10 bp of complete identity. Such patterns of homology in 3′ untranslated regions have been encountered in other genes (25, 26) but their general significance is not known.

A Southern blot analysis of DNA from animal species indicates that *PGM1* is conserved among a wide variety of vertebrates ranging from primates to birds and amphibia (Fig. 3).

***PGM1* on Human Chromosome 1.** Human *PGM1* was assigned to chromosome 1 by isozyme analysis in rodent–human somatic cell hybrids (27) and the regional localization has been refined by linkage analysis to 1p22 (28). PCR analysis using primer oligonucleotides from the 3′ untranslated region of the PGM1 cDNA confirms the assignment to 1p. Fig. 4 *Upper* shows the amplification of DNA from two hybrids, CRAB5 and CRAB4, which differ only by the presence of human chromosomes 1 and 3 in CRAB5 and their absence in CRAB4. Also shown are amplifications of DNA from TWIN19F9 and TWIN19F6, which differ only by the absence of chromosome 1 from TWIN19F6. (CRAB11, which is also shown in Fig. 4, is negative for chromosome 1 markers.) Fig. 4 also includes two hybrids HCH5 and HCH7 (20), which contain the reciprocal halves of the translocation t(1;10)(p36.31;q22.1). HCH7, which contains 1p36.1 to 1qter, is positive for the *PGM1*-amplified product whereas its counterpart HCH5 (1p36.1 to 1pter) is negative.

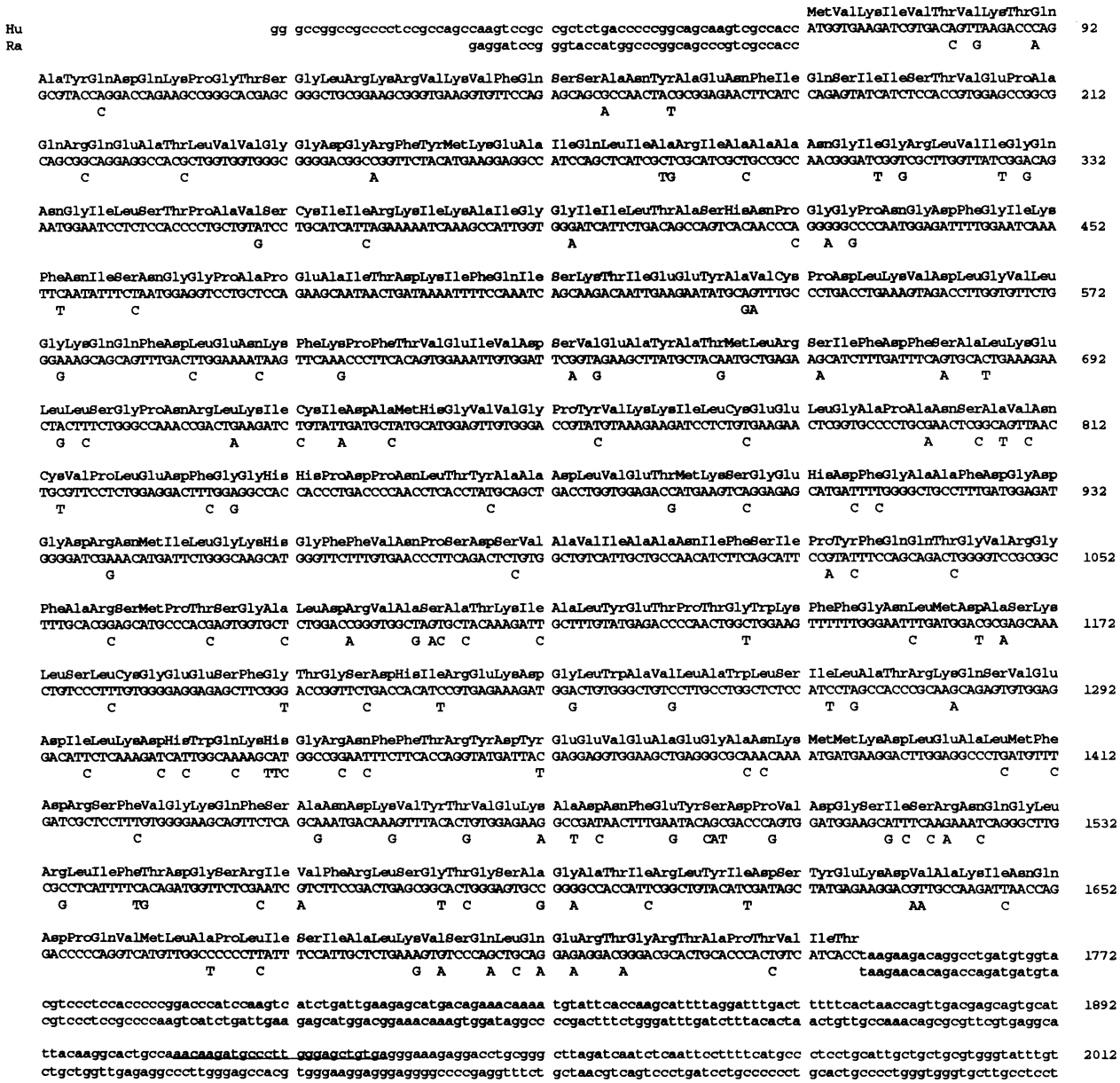

FIG. 1. Complete nucleotide sequence of the human (Hu) and rabbit (Ra) cDNAs and the derived amino acid sequence for human *PGM1*. All nucleotides are given for the untranslated regions of both species. The rabbit sequence is shown only at the positions where the amino acid coding sequences differ. Polyadenylylation signals and the 25-mer oligonucleotide primers used for PCR amplification are underlined.

The cDNA clone λHPGM1.1 was used as probe to isolate a number of genomic recombinants from Lorist B and λ2001 libraries (Y.H.E. and D.B.W., unpublished data). One of the cosmid clones LoHPGM1 was biotinylated and used as probe to determine the chromosome location by *in situ* hybridization. After hybridization the banded spreads were aligned with the fluorescent signal (Fig. 4 *Lower*) and 10 metaphase spreads were scored. The distances from the terminus to the

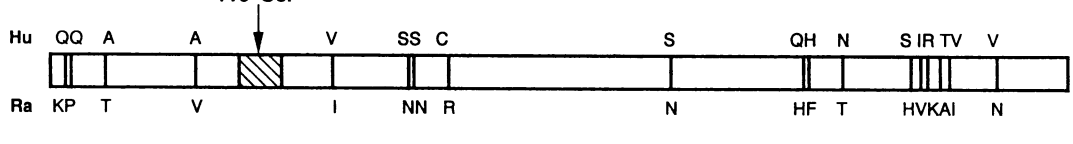

FIG. 2. Diagram of the PGM1 protein showing the positions and identity of the 18 amino acid differences between human (Hu) and rabbit (Ra) PGM1, indicated by the single-letter code and the active site (ref. 23, hatched box in figure) with Ser-116.

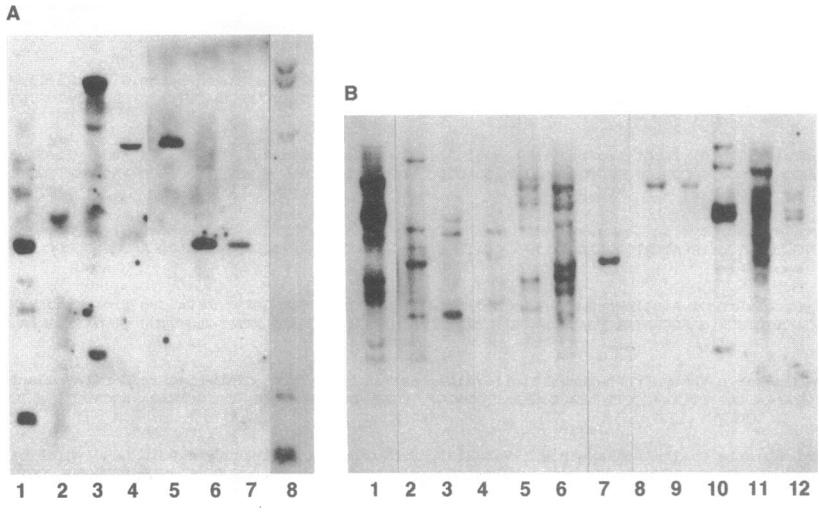

FIG. 3. Southern blot analysis of 15 μg of genomic DNA from various vertebrate species digested with *Hin*dIII (*A*) or *Taq* I (*B*) and hybridized with ^{32}P-labeled human PGM1 cDNA. The human *Hin*dIII digest was of 5 μg of DNA. Samples in *A* are as follows. Lanes: 1, zebra; 2, elephant; 3, swan; 4, tree frog; 5, bat; 6, orangutan; 7, common marmoset; 8, human. Samples in *B* are as follows. Lanes: 1, human; 2, dog; 3, cat; 4, cow; 5, sheep; 6, goat; 7, pig; 8, hamster; 9, rat; 10, mouse; 11, chicken; 12, *Xenopus*.

signal and the terminus to the centromere were measured and expressed as a proportion in each case and gave a value of 0.52 (n = 20, SD = 0.04). This indicated a location for the *PGM1* gene of 1p31.

DISCUSSION

The PGM1 protein is highly polymorphic in humans and an investigation of genetic variation at the nucleotide level should provide detailed information on the evolution of this variation. We are particularly interested to test the hypothesis of Carter *et al.* (11) developed in detail by Takahashi *et al.* (13), which proposed that reciprocal intragenic recombination between existing amino acid substitutions has been a major mechanism in the generation of structural allelic variants of *PGM1* detected by isoelectric focusing. The isolation of the complete human PGM1 cDNA sequence enables the analysis of haplotypes in genomic DNA and the identification of candidate sites of recombination. Our preliminary analysis suggests the total size of the *PGM1* gene does not exceed 34 kilobases and thus any tendency for high recombination frequency would seem to be a specific property of the *PGM1* sequence rather than a result of large target size.

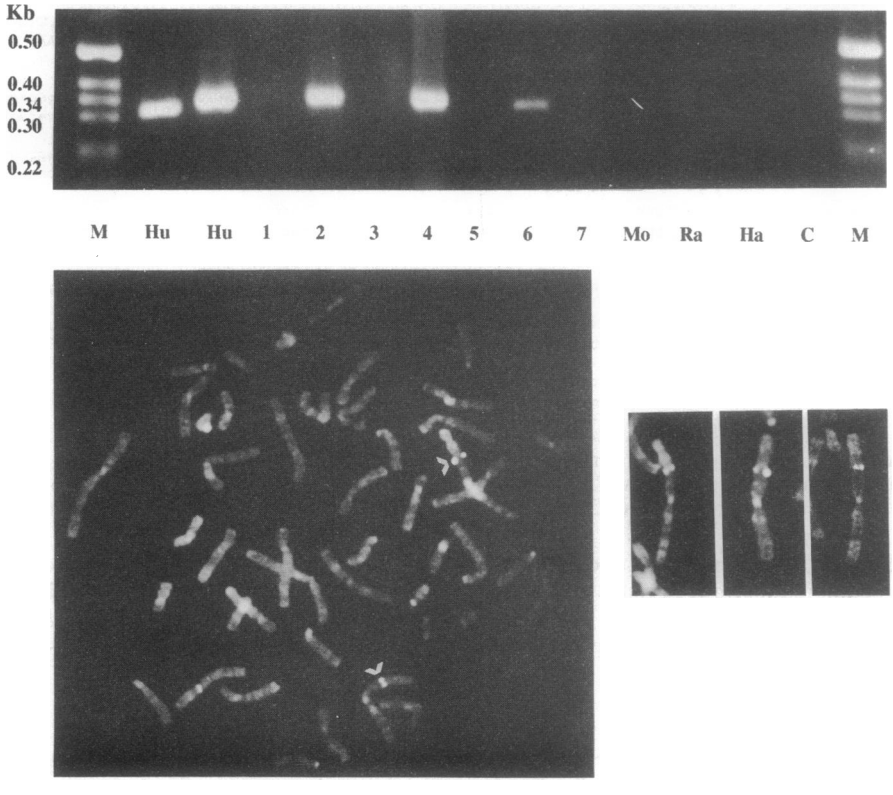

FIG. 4. (*Upper*) PCR using oligonucleotides specific for human *PGM1* and DNA from human (Hu), hamster (Ha), mouse (Mo), rat (Ra), and various rodent–human hybrid cell lines as follows. Lanes: 1, TWIN19F6; 2, TWIN19F9; 3, HCH5; 4, HCH7; 5, CRAB4; 6, CRAB5; 7, CRAB11. A control, no genomic DNA, sample (C) and size markers (M) are also shown. (*Lower*) Human metaphase R-banded chromosomes showing fluorescent *in situ* hybridization using biotinylated LoHPGM1.1 as probe. In the metaphase spread arrows indicate chromosome 1.

Interestingly, interspecific comparisons indicate less variability than might be expected if this ancient gene were highly mutable. The homology between human and rabbit PGM1 is 92% at the nucleotide level and 97% at the derived amino acid level; furthermore, a high level of sequence conservation appears to be retained throughout the vertebrate kingdom. It is noteworthy that the signal intensity from Southern blot analysis of tree-frog DNA equaled that of the orangutan and marmoset after hybridization at moderate stringency. Such sequence conservation is compatible with the view that the human PGM1 polymorphism has arisen by mechanisms other than the straightforward accumulation of point mutations (11, 13).

A computer-based comparison of the human PGM1 amino acid sequence with those of the human muscle-specific phosphoglycerate mutase PGAM2 (29) and diphosphoglycerate mutase DPGAM (30) revealed no homologies. This is in line with the finding of Ray *et al.* (23) that there are no obvious similarities between the protein sequences of rabbit muscle PGM and yeast phosphoglycerate mutase (PGAM).

No evidence for PGM1-related sequences was found either by Southern blot analysis of human genomic DNA or by *in situ* hybridization. Thus if the genes encoding human PGM2 and PGM3, assigned to chromosomes 4 and 6, respectively (2), arose by duplication from the same ancestral gene as *PGM1*, then it seems likely that less than 65% sequence homology has been preserved. Immunological data support this view; the anti-rabbit PGM1 antibodies recognize all of the human PGM1 isozymes but not those encoded by *PGM2* and *PGM3* (31) and no cross reactivity between human PGM1 and PGM2 using anti-human reagents has been demonstrated (32). Thus it may not be possible to identify cDNAs for *PGM2* and *PGM3* using PGM1 cDNA as a probe, but it is hoped that an oligonucleotide encoding the active site region will be a useful tool for this purpose.

Cloning the human *PGM1* gene has allowed the direct chromosomal localization of this locus and this has resolved some uncertainty about the exact position of this widely used genetic marker. A position for *PGM1* of 1p22 is generally accepted, although a more distal location has not been excluded (28). In the recent CEPH consortium linkage map of human chromosome 1, assembled by multipoint analysis of 58 loci, *PGM1* is positioned distal to the markers D1S22 and *ACADM* and proximal to D1S19 (33). Pakstis *et al.* (34) using linkage analysis in seven non-CEPH pedigrees, reported essentially the same order but argued that since the SRO (shortest region of overlap) for D1S19 is 1pter–p31 and for *ACADM* is 1p31 then *PGM1* cannot be in 1p22 but rather must be in 1p31 (or more distal). Our results support this view and indicate a localization of 1p31.

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