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Cloning of cDNAs for Human Phosphoribosylpyrophosphate Synthetases 1 and 2 and X Chromosome Localization of *PRPS1* and *PRPS2* Genes

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Cloned cDNAs representing the entire, homologous (80%) translated sequences of human phosphoribosylpyrophosphate synthetase (PRS) 1 and PRS 2 cDNAs were utilized as probes to localize the corresponding human *PRPS1* and *PRPS2* genes, previously reported to be X chromosome linked. *PRPS1* and *PRPS2* loci mapped to the intervals Xq22–q24 and Xp22.2–p22.3, respectively, using a combination of *in situ* chromosomal hybridization and human × rodent somatic cell panel genomic DNA hybridization analyses. A *PRPS1*-related gene or pseudogene (*PRPS1L2*) was also identified using *in situ* chromosomal hybridization at 9q33–q34. Human HPRT and PRPS1 loci are not closely linked. Despite marked cDNA and deduced amino acid sequence homology, human PRS 1 and PRS 2 isoforms are encoded by genes widely separated on the X chromosome.

INTRODUCTION

The high-energy sugar phosphate 5-phosphoribosyl 1-pyrophosphate (PP-ribose-P) is a substrate in the synthesis of purine, pyrimidine, and pyridine nucleotides (Kornberg *et al.*, 1955) and exerts a regulatory and perhaps a coordinating role in the pathways of purine and pyrimidine synthesis *de novo* (Holmes *et al.*, 1973; Becker and Kim, 1987a; Tatibana and Shigesada, 1972). Synthesis of PP-ribose-P from MgATP and ribose-5-P is catalyzed by the enzyme PP-ribose-P synthetase (PRS; ribose-5-P pyrophosphokinase; EC 2.7.6.1). Inherited superactivity of PRS has been demonstrated in association with excessive purine nucleotide and uric acid synthesis in nearly two dozen families to date. The phenotypes of affected individuals range from early adult-onset gout and/or uric acid urolithiasis to severe neurodevelopmental impairment, including sensorineural deafness, manifested in infancy (reviewed in Becker *et al.*, 1986, 1988). A variety of kinetic defects in PRS underlie enzyme superactivity (Becker *et al.*, 1987b), and more severe phenotypic expression generally parallels higher grades of aberrant enzyme function (Becker *et al.*, 1988).

Pedigree analysis of families with PRS superactivity is consistent with X chromosome-linked transmission of the trait (Sperling *et al.*, 1972; Takeuchi *et al.*, 1981). In addition, evidence has been provided (Zoref *et al.*, 1976; Yen *et al.*, 1978) for distinct normal and PRS-superactive populations of fibroblasts in cultures derived from heterozygous carrier females. Interspecies (human × Chinese hamster) hybrid cell line analysis has confirmed assignment of a human PRS structural locus (*PRPS*) to the X chromosome, permitting localization of this gene to Xq between the loci for α-galactosidase (*GLA;* Xq21.3–q22) and hypoxanthine phosphoribosyltransferase (*HPRT;* Xq26) (Becker *et al.*, 1979). Of interest has been the suggestion that the sequential reactions catalyzed by PRS and HPRT might be encoded by functionally related (Martin and Maler, 1976) and perhaps closely linked (Becker *et al.*, 1979) genes.

Tatibana and co-workers (Taira *et al.*, 1987) recently demonstrated two rat PRS cDNAs and, subsequently, two human PRS transcripts (Taira *et al.*, 1989a) which appeared to be encoded by different genes (*PRPS1* and *PRPS2*) (Taira *et al.*, 1989b). Utilizing rat (PRS 1) and human (PRS 2) cDNA probes for Southern blot and flow-sorted chromosome analyses of human and human × hamster hybrid cell panels, this group has assigned distinct X chromosome loci to human *PRPS1* and *PRPS2* genes, in the intervals Xq21–qter and Xpter–q21, respectively (Taira *et*

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al., 1989b). To further localize *PRPS1* and *PRPS2*, we performed *in situ* chromosomal hybridization and human × rodent hybrid cell line analyses using full-length coding region human PRS 1 and PRS 2 cDNAs (Roessler *et al.*, 1990). Our studies indicate that *PRPS1* is located at Xq22–q24 and is not, therefore, closely linked to *HPRT*. In addition, *PRPS2* is located at Xp22.2–p22.3, substantiating that distinct X chromosome regions code for these highly homologous isoforms of PRS.

MATERIALS AND METHODS

Cloning of Human PRS 1 and PRS 2 cDNAs

Total cellular RNA from a human B lymphoblast line and from human kidney served as templates for synthesis of cDNAs (Maniatis *et al.*, 1982) which in turn were used in conjunction with oligonucleotide primers (corresponding to rat PRS 1 cDNA sequences (Taira *et al.*, 1987)) to amplify partial human PRS cDNAs by the polymerase chain reaction (PCR), catalyzed by *Taq*I polymerase (Saiki *et al.*, 1988). Pertinent cDNAs were isolated, amplified a second time by PCR, and blunt-end cloned into phage M13 mp18 and mp19 (Yanesch-Perron *et al.*, 1985) for sequencing (Sanger *et al.*, 1977). A 399-bp cDNA highly homologous (>80% nucleotide and >98% amino acid identity) to a portion of rat PRS 1 cDNA corresponding to amino acids 184 to 317 was sequenced from the amplified lymphoblast-derived cDNA. Similarly, a 458-bp cDNA homologous (>80% nucleotide and >99% amino acid identity) to a portion of rat PRS 2 cDNA coding for amino acids 144 to 296 was sequenced from amplified kidney-derived cDNA. The amino acid sequence deduced from the human lymphoblast cDNA sequence agreed perfectly with the sequences determined for multiple peptides of purified human erythrocyte PRS (Roessler *et al.*, 1990) by gas-phase protein sequencing (Hewick *et al.*, 1981). Sequence homology between the amplified human lymphoblast and kidney cDNAs was >82%, yielding deduced amino acid sequences of >97% homology. Whenever the deduced amino acid residues diverged, the residues deduced from the kidney cDNA were identical with corresponding rat PRS 2 residues, whereas lymphoblast cDNA-deduced residues were identical with those of corresponding rat PRS 1 residues. On the basis of this homology, human lymphoblast and kidney-derived partial cDNAs were designated, respectively, hPRS 1 cDNA and hPRS 2 cDNA.

Next, hPRS partial cDNAs were ^{32}P-labeled by the oligo-primer method (Feinberg and Vogelstein, 1983, 1984) and used to probe oligo(dT)-primed cDNA libraries (prepared from the corresponding original RNA sources) using the method of *in vitro* plaque hybridization (Maniatis *et al.*, 1982). The vectors for the lymphoblast and kidney cell cDNA libraries were, respectively, λgt11 and λgt10 (Huynh *et al.*, 1984). Clones hybridizing with the labeled hPRS cDNA probes were isolated and purified by repeated plaque hybridization and grown in large cultures of the appropriate *Escherichia coli* host, and the phage DNAs were purified (Maniatis *et al.*, 1982). Restriction enzyme (*Eco*RI) digestion of the phage DNA preparations showed an array of insert sizes. Inserts of 2.0 to 2.3 kb containing hPRS 1 cDNA were cloned into M13 mp18 and 19 and were sequenced. Similarly, hPRS 2 cDNA-containing inserts ranging from 2.1 to 2.7 kb were cloned and sequenced, and appropriate restriction enzyme fragments were derived and sequenced from each of the hPRS cDNAs.

Probe Preparation

Radiolabeled hPRS cDNAs served as probes for the corresponding *PRPS* genes. *Eco*RI digestion fragments (hPRS 1 cDNA, 2.3 kb; hPRS 2 cDNA, 2.7 kb), oligo-labeled with [^{32}P]dCTP (Feinberg and Vogelstein, 1983, 1984), were used in all filter hybridization studies. After subcloning and labeling as described below, these cDNAs were also used in *in situ* chromosomal hybridization studies.

Cell Lines

Two human B lymphoblast lines (CO,46,XY; and GM01416B,48,XXXX) were examined to identify putative X-linked restriction fragments hybridizing to hPRS cDNA probes. A third human B lymphoblast line (CL,46,XX), a Chinese hamster fibroblast cell line (Wg3-h), and a mouse fibroblast line (A9) served as sources of control genomic DNA in somatic hybrid cell panel analyses. Two somatic cell line panels, the respective human X chromosome contents of which are given in Tables 1 and 2, were studied: (1) a human × Chinese hamster (Wg3-h) hybrid panel (Table 1), selected for growth in HAT medium and previously characterized with respect to expression of immunoreactive human PRS and activities of the human forms of four other enzyme markers known to map to Xq (Becker *et al.*, 1979); and (2) a human × mouse (A9) hybrid panel, also selected for growth in HAT medium and previously characterized cytogenetically and with regard to hybridization with probes for selected human Xp- and Xq-linked loci (Table 2).

DNA Preparation and Filter Hybridization

Genomic DNAs were prepared using standard methods (Maniatis *et al.*, 1982) and were digested with *Bam*HI and *Hin*dIII under conditions recommended by the manufacturer (GIBCO-BRL). After electrophoresis in 0.8% agarose gels, digests were

TABLE 1

Human × Chinese Hamster Somatic Cell Line Panel: Human X Chromosome Marker Retention and Hybridization with hPRS cDNA Probes

	Human X chromosome marker[b]					Hybridization with cDNA probe[c]	
Clone[a]	PGK-1	GLA	PRS	HPRT	G6PD	hPRS 1	hPRS 2
2D	P	P	P	P	P	+	+
Ro-13	P	P	P	P	P	+	−
Ro-52	A	A	P	P	P	+	−
Ro-43	A	A	A	P	P	−	−
Ho-13	A	A	A	P	P	−	−

[a] All clones except Ho-13 were derived from the fusion of human peripheral blood lymphocytes with Wg3-h (HPRT-deficient) Chinese hamster fibroblasts (1, 10). Clone Ho-13 is derived from fusion of Wg3-h cells with GM-97 human fibroblasts which bear a reciprocal chromosome 1 and X chromosome translocation separating the tip of Xq from the proximal portion of Xq26 (25).

[b] Except in the case of PRS, presence (P) or absence of human markers was determined by enzyme activity stain after electrophoretic separation of human and hamster forms. In the case of PRS, immunoreactivity was assayed using human PRS-specific antiserum (1).

[c] Hybridization for the respective human *PRPS* was scored as positive (+) or negative (−), the former only when one or more bands detected after autoradiography corresponded specifically to bands detected with normal human genomic DNA but not control bands detected with Wg3-h Chinese hamster genomic DNA.

transferred to GeneScreen nylon membrane filters (NEN Research Products) under alkaline conditions (Church and Gilbert, 1984). Blots were hybridized sequentially with ^{32}P-labeled hPRS 1 and hPRS 2 cDNA probes at 65°C and pH 7.2 in 500 m*M* sodium phosphate (pH 7.2) with 7% SDS and 1 m*M* EDTA prior to autoradiography. Filters were washed at 65°C to a final stringency of 40 m*M* sodium phosphate (pH 7.2), 2% SDS, 1 m*M* EDTA prior to autoradiography at −70°C for 48 h. Between hybridizations, labeled cDNA was removed from filters by washing as described (Church and Gilbert, 1984).

In Situ Chromosomal Hybridization

Human metaphase cells were prepared from phytohemagglutinin-stimulated peripheral blood lympho-

TABLE 2

Human × Mouse Somatic Cell Line Panel: Human X Chromosome Retention and Hybridization with hPRS cDNA Probes

			Hybridization with cDNA probe[b]	
Clone[a]	Human X chromosone retained	Ref.	hPRS 1	hPRS 2
33-16	q26 → qter	(34)	−	−
32-23	q22 → qter	(34)	+	−
31-1	q11 → qter	(34)	+	−
37-6	q21.05 → qter	(21)	+	−
25-8-H	p21.1 → qter	(34)	+	−
148-16	p22.1 → qter	Unpublished data (A9 × t(X; 15))	+	−
149-14	p22.1 → qter	Unpublished data (A9 × GM4618 fibroblasts)	+	−
94-7	p22.3 → qter	(9)	+	+
		(35)		
RP (human DNA)	p22.3 → qter	(35)	+	+
126-8	p22.3 → qter	(35)	+	+

[a] Clones 25-8-H, 148-16, and 149-14 have breakpoints that are currently indistinguishable both cytogenetically and with molecular probes. All three show hybridization with probes for *POLA* and *ZFX* but not with probes for *STS* or DXS278 (CRI-S232; 15). Clones 94-7 and RP, x/y translocations, have breakpoints, respectively, just proximal to and within DXS278. The breakpoint of the Xp deletion clone 126-8 is between *STS* and DXS278. *PRPS2* is located by this analysis between loci for *POLA* and *ZFX* and the locus identified by CRI-S232, an interval within which the markers *AMEL* and dic56 (DXS143) (20) also map.

[b] Hybridization was scored as described in footnote *c*, Table 1, except that the rodent parental cell source of genomic DNA was mouse A9 cells.

cytes obtained from a healthy female donor. Radiolabeled hPRS 1 and hPRS 2 cDNA probes were prepared by nick-translation of the entire cDNA in pUC18 plasmid with all four ^{3}H-labeled deoxynucleoside triphosphates to a specific activity of 1.0×10^8 dpm/μg. *In situ* hybridization was performed as described previously (LeBeau *et al.*, 1986). Metaphase cells were hybridized at 2.0, 5.0, and 25.0 ng of probe per milliliter of hybridization mixture. Autoradiographs were exposed for 11 days.

RESULTS

hPRS 1 and hPRS 2 cDNAs

The complete translated and adjacent 5′- and 3′-untranslated regions of both hPRS 1 (Roessler *et al.*, 1990) and hPRS 2 (Iizasa *et al.*, 1989; Roessler *et al.*, 1990) cDNAs were sequenced and confirmed, each in at least two independently isolated clones. These sequences appear in the EMBL/GenBank/DDBJ Nucleotide Sequence Databases under the Accession Numbers X15331 (HPR SI) and Y00971 (HPR SII). Sequence homologies between hPRS 1 and 2 cDNAs (>80%) (Roessler *et al.*, 1990) and between each hPRS cDNA and its rat counterpart (91% for PRS 1 cDNAs; 88% for PRS 2 cDNAs) are extensive throughout the 954-bp open reading frames of all four cDNAs. In contrast, homology within untranslated regions of the cDNAs is apparent only in the case of the 3′-untranslated sequences of human and rat PRS 2 cDNAs. No significant homology is present in the 5′- and 3′-untranslated regions when human PRS 1 and 2 cDNAs (Roessler *et al.*, 1990) or rat PRS 1 and 2 cDNAs (Taira *et al.*, 1987) are compared with one another.

Northern blot analysis of human poly(A)$^+$ RNA transcripts (Maniatis *et al.*, 1982) identified using labeled hPRS cDNAs containing the entire hPRS translated regions showed that hPRS 1 and hPRS 2 cDNAs identified, respectively, transcripts of 2.3 and 2.7 kb, confirming the results of Taira *et al.* (1989a). Under the usual stringent conditions of hybridization and washing, each hPRS cDNA identified only its respective mRNA.

Southern Blot Analysis

Hybridization of hPRS cDNAs with *Bam*HI- and *Hin*dIII-restricted human genomic DNA showed distinctive patterns of restriction fragments for blots probed with hPRS 1 and hPRS 2. For both restriction enzymes, some but not all of the restriction fragments identified by each probe showed distinctly greater intensity of hybridization in the lanes containing DNA from a 48,XXXX cell line than in the corresponding lanes containing an equivalent amount of 46,XY DNA. These studies thus strengthened the evidence that hPRS 1 and hPRS 2 cDNAs are encoded by distinctive genes (Taira *et al.*, 1987, 1989a) and supported assignment of human *PRPS1* and *PRPS2* genes to the X chromosome (Taira *et al.*, 1989b). The presence of genomic DNA fragments showing comparable intensity of hybridization regardless of X chromosome DNA enrichment is consistent with an additional autosomal *PRPS* gene(s) or pseudogene(s) (Taira *et al.*, 1989b).

In Situ Chromosomal Hybridization

To localize the *PRPS1* and *PRPS2* genes using an independent method, hPRS cDNA probes specific for these genes were next hybridized to normal metaphase chromosomes. This resulted in specific labeling of the X chromosome. Of 100 metaphase cells examined from the hybridization of the hPRS 1 cDNA probe, 31 (31%) were labeled on region q2 of one or both X chromosome homologs. The distribution of labeled sites on this chromosome is illustrated in Fig. 1A; of 205 labeled sites observed, 53 (25.8%) were located on this chromosome. These sites were clustered at bands q21–q24, and this cluster represented 18.5% (38/205) of all labeled sites (cumulative probability for the Poisson distribution is <0.0005). The largest number of grains was located at Xq22–q23. Specific hybridization of the hPRS 1 cDNA was also observed at a second site, namely the distal long arm of chromosome 9 (9q33–q34, 11 labeled sites, 5.4%, $P < 0.0005$) (Fig. 2). These results suggest that sequences that are homologous to *PRPS1* may be located on autosomal chromosomes. By Southern blot analysis of somatic cell hybrids and flow-sorted chromosomes, Taira *et al.* (1989b) determined that human chromosomes 7 and 9 contained *PRPS1*-related sequences, and our hybridization experiments confirm the latter finding, indicating that a *PRPS1*-related sequence (*PRPS1L2*) may be sublocalized to 9q33–q34. Similar results were obtained in a second hybridization experiment using the hPRS1 cDNA probe. Thus, by this method, the *PRPS1* gene is localized to the X chromosome, at bands q21–q24.

In hybridizations performed with the hPRS 2 cDNA probe, we observed specific labeling of the distal short arm of the X chromosome. Of 100 metaphase cells examined, 29 (29%) were labeled on region p2 of one or both X chromosome homologs (Fig. 1B). Of 174 labeled sites observed, 48 (27.6%) were located on this chromosome. These sites were clustered at band p22, and this cluster represented 22.4% (39/174) of all labeled sites ($P < 0.0005$); the largest number of grains was observed at p22.2–p22.3. Specific hybridization of the hPRS 2 cDNA probe to Xq22–q24, the site of the *PRPS1* gene, or to an autosomal chromo-

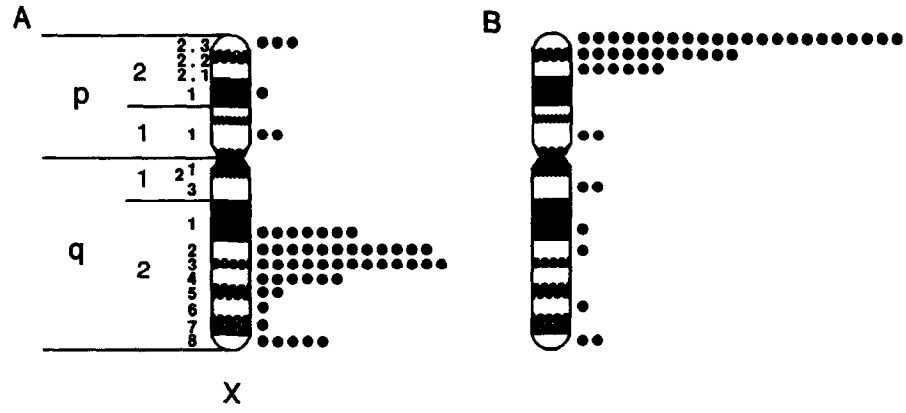

FIG. 1. Distribution of labeled sites on the X chromosome in 100 normal human metaphase cells from phytohemagglutinin-stimulated peripheral blood lymphocytes that were hybridized with the hPRS 1 (**A**) or hPRS 2 (**B**) cDNA probes. The *PRPS1*-specific probe hybridized to Xq21–q24, whereas the *PRPS2*-specific probe hybridized to the short arm of this chromosome, at band Xp22.

some was not observed. Similar results were obtained in an additional hybridization using this cDNA probe. The results of these hybridizations suggest that the *PRPS2* gene is localized to the X chromosome, at band p22, and thus, is not closely linked to the *PRPS1* gene.

Human–Rodent Hybrid Cell Line Analysis

After *in situ* hybridization studies were completed, we sought confirmation and further localization of *PRPS1* and *PRPS2* by means of hybrid cell line analysis. Hybridization of hPRS cDNAs with the genomic DNAs of unirradiated, representative human × Chinese hamster hybrid cell lines from the panel used to map a *PRPS* gene to Xq (Becker *et al.*, 1979) (Table 1; Fig. 3A) confirmed that the previously localized gene was *PRPS1* and that *PRPS1* can be assigned by this method to the region Xq22–q26 between *GLA* (Xq21.3–q22) and *HPRT* (Xq26). The results of similar studies with the human–mouse hybrid mapping panel (Table 2; Fig. 3B) were also consistent with this assignment for *PRPS1* and permitted assignment of *PRPS2* to the interval Xp22.1–p22.3, between the loci for *POLA* and *ZFX* and the locus (DXS278) identified by the probe CRI-S232 (Knowlton *et al.*, 1989).

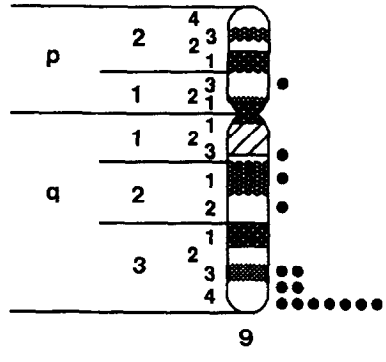

FIG. 2. Distribution of labeled sites on chromosome 9 in 100 human metaphase cells from phytohemagglutinin-stimulated peripheral blood lymphocytes that were hybridized with the hPRS 1 cDNA probe. This probe hybridized to a second site at 9q33–q34 as well as to Xq21–q24, as shown in the preceding figure.

DISCUSSION

X chromosome linkage of human *PRPS1* and *PRPS2* (Taira *et al.*, 1989b) is confirmed by these

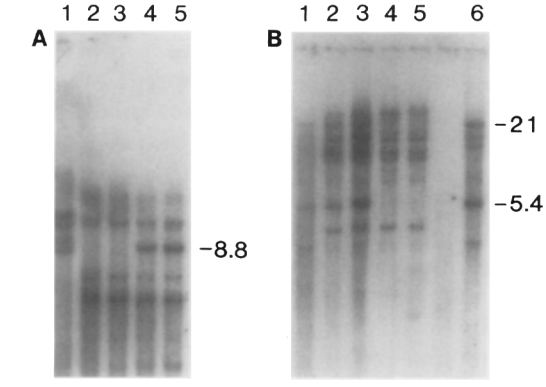

FIG. 3. Southern blot analysis of genomic DNA extracted from human, rodent, and representative human × rodent cell lines (Tables 1 and 2) and digested with *Hin*dIII (panel **A**) or *Bam*HI (panel **B**). Enzyme restricted DNA (10 μg per lane) was electrophoresed in 0.8% agarose gels, transferred to GeneScreen filters, hybridized overnight at 65°C with oligo-labeled hPRS 1 cDNA (panel **A**) or hPRS 2 cDNA (panel **B**), washed, and autoradiographed, all as described under Materials and Methods. Cell lines displayed in panel **A**: CL (human B lymphoblast), lane **1**; Wg3-h (Chinese hamster fibroblast parental), lane **2**; Ho-13, lane **3**; Ro-52, lane **4**; and 2D, lane **5**. Cell lines represented in panel **B**: CL, lane **1**; 94-7, lane **2**; 126-8, lane **3**; 148-16, lane **4**; 149-14, lane **5**, and RP, lane **6**. The sizes of bands hybridizing specifically with human × rodent cell line DNA and normal human genomic DNA but not the respective parental rodent cell genomic DNA are indicated in kilobases. Although, omitted from panel **B**, mouse (A9) fibroblast genomic DNA did not demonstrate 21- or 5.4-kb bands.

studies which also permit more precise regional localization of these genes. The results of hybridization studies utilizing human × Chinese hamster hybrid cell lines previously employed to map a human *PRPS* structural gene by immunochemical and enzyme activity assays (Becker *et al.*, 1979) clearly demonstrate that the locus so mapped was *PRPS1*. *In situ* chromosomal hybridization and analysis of additional human–rodent hybrid cell lines support assignment of *PRPS1* to the interval Xq22–q24, a region consistent with the previous localization of this marker between *GLA* and *HPRT*. In additional studies not shown here, hybridization analysis of enzyme-restricted genomic DNA after pulsed-field gel electrophoresis showed that *PRPS1* does not reside on a common restriction fragment with *HPRT*. Linkage between *PRPS1* and *HPRT*, previously demonstrated by hybrid cell line analysis (Becker *et al.*, 1979), clearly does not extend to these more sensitive methods of analysis.

PRPS2, a gene encoding a cDNA with striking homology to hPRS 1 cDNA, maps to a region at the opposite end of the X chromosome, namely Xp22.2–p22.3. Although hPRS 2 cDNA identifies a distinct 2.7-kb transcript expressed in a wide range of tissues (Taira *et al.*, 1989a), no precise information is available concerning the functional properties of the putative protein product whose amino acid sequence is predicted to be >97% homologous to hPRS 1. It has been suggested that rat PRS 1 and PRS 2 are subunits of PRS (Taira *et al.*, 1987); we have, however, found no evidence of a protein with the predicted primary structure of hPRS 2 as even a minor contaminant of purified human erythrocyte PRS during protein sequencing studies which have identified >75% of the amino acid sequence of hPRS 1 (Roessler *et al.*, 1990). In addition, purified erythrocyte hPRS 1 retains substantial catalytic activity through the final purification step (M. A. Becker, unpublished results), indicating that this isoform, at least, is enzymatically active in the absence of hPRS 2. Delineation of the interactions of human *PRPS1* and *PRPS2* genes and their products will await an understanding of the range and regulation of expression of these two loci as well as identification of hPRS 2 and its enzymatic and regulatory properties. Furthermore, which X chromosome-linked *PRPS* is primarily altered in each family with inherited PRS superactivity remains to be established definitively. Direct comparison of the sequences and expression of the hPRS 1 and hPRS 2 cDNAs of normal and affected persons should clarify this problem.

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