Structure of the Rat PRPS1 Gene Encoding Phosphoribosylpyrophosphate Synthetase Subunit I

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Phosphoribosylpyrophosphate (PP-Rib-P) synthetase (EC 2.7.6.1) subunit I gene (PRPS1) is constitutively expressed in various tissues (Taira, M., Iizasa, T., Yamada, K., Shimada, H., and Tatibana, M. (1989) Biochim. Biophys. Acta 1007, 203-208). We report here the exon-intron organization and the transcription promoter sequence of rat PRPS1 gene. This gene has 22 kilobases and is split into 7 exons ranging in size from 99 to 251 base pairs (bp), except for exon 7 (1008 bp). A putative PP-Rib-P binding site is encoded in exon 5. The exon-intron boundaries are similar to the consensus sequences for mammalian introns. 3' UTR and primer extension assays with the use of RNA from rat Yoshida ascites sarcoma cells led to the identification of four possible transcription start points closely spaced between 126 and 129 bp from the ATG initiation codon. In the upstream region from the transcriptional start sites, we observed a TATA-like sequence (TAAATTAAT) at nucleotides -28, a CCAAT element (AGCCAACT) at nucleotides -80, and three GC boxes (putative Sp1-binding sites) at nucleotides -103, -43, and -10. A comparison of the promoter region for PRPS1 with those of other housekeeping genes revealed a homology resembling that of the β-actin gene.

Phosphoribosylpyrophosphate (PP-Rib-P)\(^{1}\) synthetase (ATP:d-ribose-5-phosphate pyrophosphotransferase, EC 2.7.6.1) catalyzes a crucial step required for the biosynthesis of purine, pyrimidine, and pyridine nucleotides.

\[ \text{d-Ribose 5-phosphate} + \text{ATP} \rightarrow \text{PP-Rib-P} + \text{AMP} \]

Since PP-Rib-P is an essential substrate for supply of a ribose moiety to form nucleotides, this reaction connects the major metabolic pathways of the pentose phosphate shunt and nucleotide synthesis. Thus, PP-Rib-P synthetase is an indispensable housekeeping enzyme. It would be expected that such a reaction would be subject to strict metabolic control, with regard to both enzymatic activity and gene expression.

PP-Rib-P synthetase has been purified from bacteria (Switzer and Gibson, 1978; Hove-Jensen et al., 1986) and mammalian tissues (Fox and Kelley, 1971; Roth et al., 1974; Kita et al., 1989). The enzymatic activity is modulated by many effectors: Mg\(^{2+}\) and inorganic phosphate as activators; and ADP, 2,3-bisphosphoglycerate, or GDP as competitive or noncompetitive inhibitors (Becker et al., 1979). The enzyme is an oligomeric complex composed of about 25 kDa subunits, and we reported that the rat liver enzyme exists as complex aggregates of 34-, 38-, and 40-kDa components, the 34-kDa species being the catalytic subunit (Kita et al., 1989).

During rat cDNA cloning experiments and amino acid sequencing of the purified enzyme, we noted the presence of two distinct types of the 34-kDa subunits, PRS I and PRS II (Taira et al., 1987; Kita et al., 1989). The predicted proteins (both 317 residues) varied by only 13 amino acids. The nucleotide sequences of the two cDNAs suggested that PRS I and PRS II mRNAs were encoded by two distinct genes, designated as PRPS1 and PRPS2, respectively. Human gene mapping showed that PRPS1 and PRPS2 were located in the different regions of the X chromosome (Taira et al., 1989a).

Either PRPS1 or PRPS2, or both mRNAs were detected in almost all tissues of the rat, and levels increased after partial hepatectomy (Taira et al., 1987, 1989b).

We report here the isolation and structural analysis of the entire rat PRPS1 gene as well as the determination of transcriptional start sites. The coding region is contained within a 22-kilobase (kb) DNA segment and is divided into 7 exons. The sequence of the promoter region suggests the existence of a TATA box, a CCAAT element, and Sp1-binding sites.

**EXPERIMENTAL PROCEDURES**

Isolation of Genomic Clones—An EMBL3 genomic library was constructed from female Sprague-Dawley rat liver DNA partially digested with MboI. This library was screened by hybridization with nick-translated fragments of rat PRPS1 and PRPS2 cDNAs (SacI 1.25 kb and BglII/HindIII 1.5 kb, respectively; Taira et al., 1989b) as probes and washed in 2 x SSC (0.3 M NaCl, 0.03 M sodium citrate, pH 7.0) and 0.1% sodium dodecyl sulfate at 68 °C. DNA samples were isolated from recombinant phages amplified by growing on a 0.8% agarose plate. They were mapped by double or partial digestion and by Southern blot analysis. DNA fragments obtained from the digestes were subcloned into pUC118, pUC119 (Takara Shuzo, Kyoto), or pGEM-2 (Promega Biotec, Madison) for further analysis.

DNA Sequencing—Restriction fragments were subcloned into either M13mp18 or -19. Nucleotide sequences were determined by the dyeoxy chain termination method (Sanger et al., 1977) using deoxy-dazaguanosine triphosphate or dITP (Sequenase, United States Biochemical Corp., Cleveland) as a substrate instead of dGTP to avoid compression of the sequencing bands.

RNA Isolation—Poly(A)\(^{+}\) RNA was prepared from rat Yoshida ascites sarcoma (YS) cells by extraction with guanidinium isothiocyanate followed by oligo(dT)-cellulose chromatography (Maniatis et al., 1982).

**Primary Synthesis and 5' End Labeling**—Oligonucleotide primers were synthesized with a 380B DNA synthesizer (Applied Biosystems Inc., Foster City: Primer 1, CCGGCTGAAAGTTTGTATCGCG

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(an anti-sense sequence from nucleotides +133 to +156 adjacent to SacI site); Primer 2, CTAGCTGAAGCAATCTTG; Primer 3, CTCTTGGCTCCGCAGC; and Primer 4, GCTGACACTTGTGGTAC. Primer 1 was 5' end-labeled with [γ-32P]ATP (ICN Biomedicals Inc.) and T4 polynucleotide kinase (Takara) at 37 °C for 30 min (a specific activity of 3 × 10^6 cpm/μg). Unincorporated [γ-32P]ATP was removed with the use of a Quick Spin Column (Sephadex G-25, Boehringer Mannheim).

**S1 Nuclease Mapping**—A genomic DNA fragment (RsaI/SacI 382 bp; positions -226 to +156) covering the putative transcription initiation site (see Figs. 2 and 3) was heat-denatured and dephosphorylated with bacterial alkaline phosphatase (Takara). To obtain a single-stranded DNA prior to labeling a recessed 5' end at the SacI site, the DNA fragment was strand-separated by electrophoresis on a 7 M urea, 5% polyacrylamide gel layered on 7% gel (49:1). The two single-stranded fragments were visualized with ethidium bromide and eluted from the gel. One of these, which did not hybridize to 32P-end-labeled Primer 1, was used as a S1 probe. The probe was end-labeled with [γ-32P]ATP and T4 polynucleotide kinase to a specific activity of 9 × 10^6 cpm/μg. Unincorporated [γ-32P]ATP was removed with the use of a Quick Spin Column. S1 nuclease protection analysis with the use of poly(A)+ RNA from YS cells was carried out as follows (Berk and Sharp, 1977). The poly(A)+ RNA (5 μg) was mixed with 6–30 × 10^4 cpm of probe in 50 μl of a solution comprised of 56% deionized formamide, 0.4 M NaCl, and 1 mM EDTA. The hybridization mixture was incubated first at 90 °C for 5 min and then at 56 °C for 12 h. Nucleic acids were digested with either 250 or 500 units of S1 nuclease (Takara) in 500 μl of a solution containing 50 mM sodium acetate, pH 4.6, 280 mM NaCl, and 4.0 mM ZnSO4 at 30 °C for 90 min. The samples were ethanol-precipitated and electrophoretically separated on a 7 M urea, 5% polyacrylamide-sequencing gel. Radioactive bands were identified by autoradiography with Kodak XAR-5 film, and their sizes were estimated by comparison with the genomic nucleotide sequence ladder. Sequencing reactions were carried out using Primer 1 labeled at the same position as the S1 probe.

**Primer Extension Analysis**—Three pmol of Primer 1 (8 × 10^6 cpm)
FIG. 2. Partial nucleotide sequence of the rat PRPS1 genomic gene. DNA sequences corresponding to exons and the flanking regions are in capital letters; intron sequences are in lowercase letters. Nucleotide position 1 is assigned to the 5' end of the transcription initiation sites as determined by S1 nuclease mapping and primer extension analysis (see Fig. 3), and residues preceding it are indicated by negative numbers. The last nucleotide in every line is numbered on the right. The restriction endonucleases are indicated above the nucleotide sequence.
was hybridized to poly(A)\(^+\) RNA (5 \(\mu\)g) in 5 \(\mu\)l of a solution containing
the Sl mapping and the primer extension. The position of the longest
primer extension products, the 5' end of the Sl probe was prepared
was annealed to 5 \(\mu\)g of YS cell poly(A)' RNA (lane 5) or yeast tRNA
The "P-labeled Primer 1, complementary to positions +133 to +156,
hybridized with 5 \(\mu\)g of poly(A)+ RNA in a final volume of 25 \(\mu\)l
for 3 min and transferred to 37 °C for 2 h. The primer extension
reactions were carried out at 37 °C for 60 min in 50
Tris-Cl, pH 7.4, 100 \(\mu\)M NaCl. Mixtures were heated at 90 °C
for 3 min and transferred to 37 °C for 2 h. The primer extension
reactions were carried out at 37 °C for 60 min in 50 mM Tris-Cl, pH
8.3, 8 mM MgCl\(_2\), 30 mM KCl, 4 mM sodium pyrophosphate, 4 mM
dithiothreitol, 500 \(\mu\)M of each deoxynucleotide triphosphate, 25 units
of reverse transcriptase (Boehringer Mannheim), 32 units of RNase
inhibitor (Promega Biotec), and 3 pmol of "P-labeled Primer 1
hybridized with 5 \(\mu\)g of poly(A)\(^+\) RNA in a final volume of 25 \(\mu\)l
(Agarwal et al., 1981). The mixture was passed through a Quick Spin
Column and ethanol-precipitated. The extension products were ana-
yzed by electrophoresis on a 7 m urea, 5% polyacrylamide gel as
described above for S1 nuclease mapping.

**RESULTS AND DISCUSSION**

Isolation of Genomic Clones—About 2 \(\times\) 10\(^5\) recombinant
phages from a female rat liver genomic library were screened
using "P-labeled rat PRPSI and PRPS2 cDNA fragments as
probes. Thirteen positive recombinant phages were obtained;
the PRPS1 cDNA probe was strongly hybridized to 12 clones,
whereas the PRPS2 probe only hybridized to one. The former
12 clones were subjected to further analysis by restriction
enzyme mapping and Southern blot analysis. Nine out of 12
clones comprised an overlapping set of clones spanning about
44 kb and the entire PRPS1 gene (Fig. 1A). Their partial
nucleotide sequences were identical to that of rat PRPS1
cDNA, showing that this gene is rat PRPS1. To define posi-
tions and boundaries of the PRPS1 exon blocks, the restric-
tion fragments hybridized with the cDNA probe were sub-
cloned (Fig. 1A) and their sequences determined (Fig. 1B).
The restriction maps and the exon/intron organization of the
PRPS1 gene is shown in Fig. 1A. Thus, 7 exons were distrib-
uted over about 22 kb. BamHI fragments of 10 and 12 kb
were hybridized to poly(A)+ RNA (lanes 6 and 5) or yeast tRNA
(Sl probe only hybridized to one. The former

exons 4 and 5. A highly conserved region
encoded in two exons 4 and 5. A highly conserved region
encoded in two exons 4

Location of Transcription Initiation Site—To determine the
5' end of the PRPS1 mRNA, S1 nuclelease protection assays
were performed with poly(A)\(^+\) RNA isolated from YS cells,
which highly expressed PRPS1 mRNA (Taira et al., 1989b).

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**Ref.** Rat PP-Rib-P Synthetase Subunit I Gene (PRPSI)
values are in good agreement with findings in case of the Sl sites (Breathnach and Chambon, 1981); 2) TAATTTAAT sequence is observed, which is not similar to the canonical TATA box, as described below in YS cells.

For purposes of confirmation, primer extension analysis was performed. A major extension product of 148 bases was obtained upon reverse transcription with Primer 1 in addition to minor extension products of 146, 145, and 144 bases. These values are in good agreement with findings in case of the Sl site. Therefore, the 5' end of the TATA sequence suggested by the S1 mapping is defined as +1, which locates 129 bases upstream from the translation initiation codon ATG.

Predicted Promoter Elements—Several possible promoter elements were identified in the 5'-flanking region (Fig. 2). The TAATTTAAT sequence at positions -30 to -21 is embedded in the GC-rich region (from -43 to -5). Though this sequence is not similar to the canonical TATA box, TATA(A/T)A(A/T)A (Breathnach and Chambon, 1981), it seems to serve as the TATA box for the following reasons. 1) The TAATTTAAT sequence is found in reported TATA-like sequences in several genes lacking the TATA box (Nudel et al., 1983; Kost et al., 1983); CCAAT boxes, TATA-like regions, and TATA boxes embedded in GC-rich regions correspond to each TATA sequence are boxed. Asterisks, identical residues among the three sequences. Bars, GC- or TC-rich regions.

The DNA fragment (Real/Sacl 382 bp) labeled at the SacI site, a position 24 bases downstream from the translation initiation codon, was used as a probe. Protected fragments were estimated by comparison with the genomic nuclease sequence ladder initiated from Primer 1, which was labeled at the same position as the S1 probe (Fig. 3). A major protected fragment of 147 bases and minor protected fragments of 148, 146, and 145 bases were detected (lanes 3 and 4). These results suggested that transcription of the PRPSI gene started from the tetranucleotide TCTA at position +1 through +4 (as described below) in YS cells.

The GC boxes (GGGCGG, putative Sp1-binding sites) were found at positions -102 (reverse orientation), -43 (reverse orientation), and -10, all of which match perfectly the Sp1 consensus sequence, G/(T)/G/GCGG/(T)/G/A/(G/A)/(C/T) (Briggs et al., 1988), except A at the 3' end of the site at position -10. It is tempting to speculate that these Sp1 sites may facilitate the recognition of TAAATTTAAT as has been suggested for the SV40 early promoter region containing six Sp1 sites and a downstream "weak" TATA box of TATTTT sequence (Vigieron et al., 1984; Mathis and Chambon, 1981).

Thus, the rat PRPSI gene seems to possess three kinds of fundamental promoter elements which may play a role in expression of this housekeeping gene. In this regard, we compared promoter sequences among other housekeeping genes and found that the sequence of rat PRPSI gene is homologous to those of rat and chicken β-actin genes (Nudel et al., 1983; Kost et al., 1983); CCAAT boxes, TATA-like regions, and TATA boxes embedded in GC-rich regions correspond to each segment of this sequence (Fig. 4). The significance of this homology as well as activities of PRPSI promoter elements remain to be clarified.

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