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. S1 nuclease 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 128 and 129 bp from the ATG initiation codon. In the upstream region from the transcriptional start sites, we observed a TATA-like sequence (TAATTTAAT) at nucleotides -28, a CCAAT element (AGCCCAATC) 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) 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.

**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 BstEII/HincII 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. DNAs 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 digests 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-7-deazaguanosine triphosphate or dGTP (Sequenase, United States Biochemical Corp., Cleveland) as a substrate instead of dGTP to avoid compression of the sequencing bands.

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

During rat cDNA cloning experiments and amino acid sequencing of the purified enzyme, we noted the presence of two different 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 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.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) J05251.

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The abbreviations used are: PP-Rib-P, 5-phosphoribosyl 1-pyrophosphate; PRS I and PRS II, phosphoribosylpyrophosphate synthetase subunit I and II, respectively; bp, base pair(s); kb, kilobase(s); YS cells, rat Yoshida ascites sarcoma cells.

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Rat PP-Rib-P Synthetase Subunit I Gene (PRPS1) (an anti-sense sequence from nucleotides +133 to +156 adjacent to SacI site); Primer 2, CTAGCTGAAGCAATCTTG; Primer 3, CTCTTGGCTCCGCCAGC; 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 9 × 10^6 cpm/μg). Unincorporated [γ-32P]ATP was removed with the use of a Quick Spin Column (Sephadex G-25, Boehringer Mannheim).

SI 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 Sl 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. SI 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 30 °C for 5 min and then at 56 °C for 12 h. Nucleic acids were digested with either 250 or 500 units of SI 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 SI 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 (a) 5' to 3'.
...was hybridized to poly(A)+ RNA in 5 ml of a solution containing 10 mM Tris-Cl, pH 7.4, 100 mM NaCl. Mixtures were heated at 90 °C for 2 min and then quickly chilled in an ice bath for 2 h. The primer extension reactions were carried out at 37 °C for 30 min in 50 mM Tris-Cl, pH 8.3, 8 mM MgCl₂, 30 mM dithiothreitol, 500 units of reverse transcriptase (Boehringer Mannheim), 32 units of RNase inhibitor (Promega Biotec), and 3 pmol of 32P-labeled Primer 1, complementary to positions +133 to +156, annealed to poly(A)+ RNA (5 pg) in 5 ml of a solution containing 10 mM Tris-Cl, pH 7.4, 100 mM NaCl. Mixtures were heated at 90 °C for 2 min and then quickly chilled in an ice bath 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 MgCl₂, 30 mM dithiothreitol, 500 units of reverse transcriptase (Boehringer Mannheim), 32 units of RNase inhibitor (Promega Biotec), and 3 pmol of 32P-labeled Primer 1, complementary to positions +133 to +156.

To define positive recombinant plasmids, nucleotide sequences were identical to that of rat PRPSl gene (Fig. 1A). Their partial nucleotide sequences were determined (Fig. 1B). Thirteen positive recombinant phages were obtained; 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 genomic gene. BamHI fragments of 10 and 12 kb corresponded to the 2 bands out of 8 obtained from the genomic Southern blot analysis in previously published work (Taira et al., 1985). A polypyrimidine tract preceded the 3' ends of all introns. Positive hybridizing bands were identified by the 3' ends of all introns. Positive hybridizing bands were identified by the genomic Southern blot analysis in previously published work (Taira et al., 1985). A polypyrimidine tract preceded the 3' ends of all introns. 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values are in good agreement with findings in case of the Sl protection assay. Therefore, the 5' end of the TGTA sequence to minor extension products of 146, 145, and 144 bases. These elements were identified in the 5'-flanking region (Fig. 2). The 5' end of the TAATTTAAT sequence locates at -30, seems to serve as the TATA box for the following reasons. 1) this sequence is not similar to the canonical TATA box, embedded in the GC-rich region (from -43 to -5). Though from the tetranucleotide TGTA at position +1 through +4 was performed. A major extension product of 148 bases was suggested by the Sl mapping is defined as +1, which locates the mRNA start site of this 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/T)A (Breathnach and Chambon, 1981), it seems to serve as the TATA box for the following reasons. 1) The 5' end of the TAATTTAAT sequence locates at -30, which corresponds to the general location of the TATA box at positions -34 to -26 (Breathnach and Chambon, 1981); 2) the mRNA start site of this PRPS1 gene was defined in a narrow region rather than in multiple regions; multiple initiations were noted in several genes lacking the TATA box (Yamaguchi et al., 1987); and 3) the ATTATA motif in the TAATTTAAT sequence is found in reported TATA-like sequences of 14 genes including SV40 early promoter (TAATTAT) (Mathis and Chambon, 1981) out of 168 (Bucher and Trifonov, 1986).

A CCAAT sequence was found at the -80 position. This promoter sequence generally occurs between positions -70 and -80 in eukaryotic genes (Breathnach and Chambon, 1981). Furthermore, the sequence of CCGGTTCACCGCA ATCCCGGA (positions -89 to -71) resembles a CCAAT consensus sequence, (C/T)AG(C/T)NNN(A/G)RCCAAT-CNNNR, which is bound to a CCAAT binding protein CP2 (Chodosh et al., 1988).

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/A)GGCG(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 putative Sp1 sites may facilitate the recognition of TAATTTAAT as has been suggested for the SV40 early promoter region containing six Sp1 sites and a downstream "weak" TATA box of TATTTTAT sequence (Vigneron et al., 1984; Mathis and Chambon, 1981).

Thus, the rat PRPS1 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 PRPS1 gene is homologous to those of rat and chicken β-actin genes (Nudel et al., 1983; Kost et al., 1983); CCAAT boxes, TC-rich regions, and TATA boxes embedded in GC-rich regions correspond to each (Fig. 4). The significance of this homology as well as activities of PRPS1 promoter elements remain to be clarified.

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