SHORT COMMUNICATION

Rat Hypoxanthine Phosphoribosyltransferase cDNA Cloning and Sequence Analysis

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We have determined the nucleotide sequence of the rat *hprt* (hypoxanthine phosphoribosyltransferase; EC 2.4.2.8.) mRNA coding region and of adjacent, untranslated 5′ and 3′ mRNA, and we have designed an oligonucleotide primer pair for efficient PCR amplification of the rat *hprt* coding region. These sequence data and rat-specific primer pair will aid workers interested in coupling well-developed rat toxicologic and carcinogenicity bioassays with quantitative and molecular analyses of somatic mutation induction in rat cells *in vivo* and *in vitro*.

Hypoxanthine phosphoribosyltransferase (HPRT; EC 2.4.2.8.) plays an important role in purine nucleotide salvage in mammalian cells and is the best-characterized of a larger group of phosphoribosyltransferases that utilize α-D-5-phosphoribosyl-1-pyrophosphate (PRPP) in the synthesis of purines, pyrimidines, histidine, and tryptophan (Musick, 1981). HPRT plays an essential role in purine metabolism in lower organisms as well. The malarial parasite *Plasmodium falciparum* and visceral leishmaniasis parasite *Leishmania donovani*, for example, cannot synthesize purines *de novo* and rely on parasite-encoded HPRTs for purine production during portions of their life cycles. The essential role of these HPRTs in the parasite life cycle makes them attractive targets for antiprotozoal chemotherapy (Wang, 1984).

Mammalian *hprt* genes are X-linked and, by virtue of X-chromosome inactivation, are functionally hemizygous in somatic cells. There are simple purine analogue selections for HPRT-deficient mutants, and a wealth of restriction maps, probes, and nucleotide sequence data exist for several mammalian *hprt* genes (reviewed in Stout and Caskey, 1989). These biological and methodologic advantages have led to wide use of the *hprt* locus for quantitative and molecular mutation analyses in mammalian somatic cells. We have determined the nucleotide sequence of the rat *hprt* mRNA coding region, and have designed a pair of rat *hprt*-specific oligonucleotide primers to aid workers interested in coupling rat toxicologic and carcinogenicity bioassays with molecular analyses of somatic mutation induction in rat cells *in vivo* and *in vitro*.

Our strategy for isolating the rat *hprt* cDNA consisted of direct PCR amplification and sequencing of *hprt* cDNA sequences from two independent rat cDNA libraries, followed by specific amplification and sequencing of a *hprt* cDNA from rat liver epithelial cell RNA. We anticipated that rat *hprt* would show substantial nucleotide sequence homology with other mammalian *hprt* coding regions and thus chose for initial amplifications two human *hprt* cDNA primers that have extensive 3′ homology with both the mouse and hamster *hprt* coding regions (441(−) and 461(+); Fig. 1). These consensus primers were used with cloning vector- and additional *hprt*-specific primers to amplify and sequence the rat *hprt* cDNA coding and flanking regions. The cDNA libraries were a λgt11 rat liver cDNA library prepared from adult male Sprague–Dawley rat liver (Clontech No. RL1023b), and a λZAPII neonatal rat smooth muscle cDNA library prepared from cultured aortic medial smooth muscle cells from 12-day-old Wistar–Kyoto rats (kindly supplied by Drs. Ceci Giachelli and Steve Schwartz, Department of Pathology, University of Washington, Seattle, WA). Both libraries were prepared by oligo(dT)-primed reverse transcription of poly(A^+) mRNA. The DNA sequence of both strands of the rat *hprt* cDNA fragments amplified from each library was determined by direct dideoxy chain terminator sequencing using modified T7 DNA polymerase (Sequenase). A portion of 3′ untranslated cDNA sequence was also derived from cloned amplification products. The nucleotide sequences of the 657-bp rat *hprt* coding region and of 98 bp of 5′ and 98 bp of 3′ untranslated sequence amplified from each library were identical. We did not determine the nucleotide sequence of an additional 400 bp of untranslated 3′ sequence included in the library amplification prod-

Sequence data from this article have been deposited with the *EMBL*/GenBank/DDBJ Data Libraries under Accession No. M63983.

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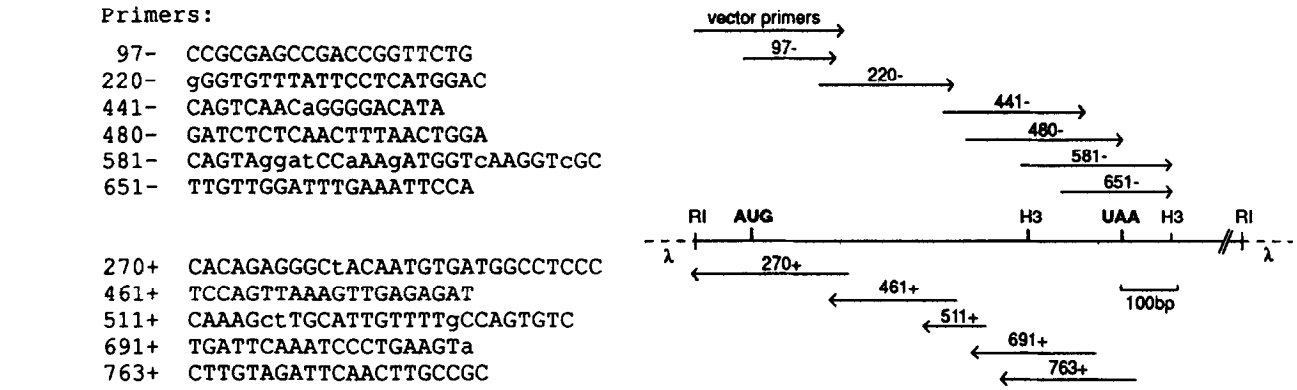

FIG. 1. Strategy for PCR amplification and direct sequencing of the rat *hprt* cDNA. The rat *hprt* cDNA is shown as a solid line flanked by λ cloning vector sequences (dotted line). The positions of start (AUG) and stop (UAA) codons, of cDNA *Hin*dIII (H3) sites used for subcloning and of cloning vector *Eco*RI (RI) cDNA insertion sites are indicated above the sequence. The H3–RI crosshatch region contains an additional 400 bp of untranslated 3′ sequence. Arrows above and below the cDNA sequence indicate the direction and extent of nucleotide sequence determined from specific primers shown to the left. Primer sequences are listed 5′ to 3′. Nucleotides that are mismatched with the rat *hprt* cDNA sequence are indicated in lower case. One primer (691(+)) worked as an efficient PCR primer despite a mismatched 3′-OH terminal nucleotide. Primer numbers indicate the position of the primer 3′ nucleotide on the human HPRT cDNA sequence of Jolly *et al.* (5). "Plus" primers are complementary to the sense or mRNA-equivalent cDNA strand, while "minus" primers are complementary to the antisense cDNA strand. Primers 97(−) and 763(+) were specifically designed for rat *hprt* cDNA coding region amplification.

ucts. We subsequently confirmed this cDNA sequence by synthesizing a rat *hprt* cDNA-specific primer pair (97(−) and 763(+); Fig. 1) that was used to amplify the *hprt* coding region from Fisher F344 NRLM rat liver epithelial cell (McMahon *et al.*, 1986) RNA.

A comparison of the nucleotide and deduced amino acid sequences of rat HPRT with other mammalian HPRTs revealed coding region nucleotide sequence identities of 93.3% (human), 94.4% (hamster), and 96.7% (mouse), and 15 different amino acid substitutions between rat and human, mouse, or hamster HPRTs. Ten substitutions were observed between rat and human or hamster HPRTs, and 4 substitutions between rat and mouse HPRTs (Table 1). These amino acid substitutions are located in the N- and C-terminal portions of HPRT and flank a 109-residue region (HPRT residues 42–150) that is believed to contain a hypoxanthine binding site and the N-terminal portion of a PRPP binding site (Hershey and Taylor, 1986; Davidson *et al.*, 1989). Of the 15 residues that vary among mammalian HPRTs only two, ser_7 (gly_7 in human HPRT) and leu_{41} (val_{41} in hamster) have been identified as the sites of mutations in Lesch–Nyhan or gout patients (gly_7 to asp_7 in $HPRT_{Gravesend}$, and leu_{41} to pro_{41} in $HPRT_{Detroit}$; Davidson *et al.*, 1989, 1991).

We anticipate that the rat *hprt* cDNA sequence data and primer pair reported here will be useful to workers interested in using the rat *hprt* locus for somatic mutation analyses. The sequence data reported here should also aid analyses of the enzymology and structural biology of HPRT and other phosphoribosyltransferases. Both wildtype and mutant human HPRTs can be selected by complementation and expressed as enzymatically active proteins in *gpt*/*hpt*-deficient *Escherichia coli* (see, e.g., Free *et al.*, 1990).

TABLE 1

HPRT Amino Acid Substitutions between Mammalian Species

Rat residue and position number	Mouse	Hamster	Human
Ser_2	Pro	Ala	Ala
Leu_4	Arg	Arg	Arg
Ser_7	—	—	Gly
Ala_{29}	—	Val	—
Lys_{34}	—	—	Arg
Leu_{41}	—	Val	—
Lys_{151}	—	—	Arg
Gln_{152}	—	Arg	—
Ser_{154}	—	Asn	Asn
Pro_{155}	—	Leu	—
Ser_{169}	—	—	Pro
Arg_{175}	—	—	Lys
His_{198}	Tyr	Tyr	Tyr
Val_{205}	—	Ile	—
Ser_{211}	Thr	Thr	Thr

Note. (—) indicates that the residue at this position is identical to that found in rat HPRT. The EMBL/GenBank Accession Nos. of HPRT sequences used for this comparison are: mouse (J00423 and K01514); hamster (J00060); and human (J00205).

This approach should facilitate systematic analyses of the contribution of different HPRT residues to enzyme stability, activity, and substrate specificity. A better understanding of how HPRT structure determines function would be intrinsically interesting and may have practical utility given the central role these enzymes play in purine, pyrimidine, and amino acid biosynthesis, in inherited diseases and in protozoal infection.

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