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Organization of rat uricase chromosomal gene differs greatly from that of the corresponding plant gene

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The rat chromosomal gene for uricase was cloned. The gene spans more than 20 kilobases and the coding region is divided into 8 exons by 7 introns. The organization of the rat uricase gene is so greatly different from that of the soybean gene that the difference may not have been caused only by the removal of some ancestral introns during the period of widely separated evolution.

Uricase; Urate oxidase; Nucleotide sequence; Chromosomal gene; Gene organization

1. INTRODUCTION

The extent of degradation of purine differs among species and the enzymes responsible for the degradation have been lost during evolution [1]. Uricase (urate oxydase; EC 1.7.3.3) is a copper-containing oxidase responsible for the hydrolysis of uric acid to allantoin in the purine degradation pathway. Humans and some primates lack uricase and excrete uric acid as the end-product of purine degradation [2]. Other mammals have uricase and the enzyme is known to be localized in the peroxisome where it forms large electron-dense paracrystalline cores [3].

The amino acid sequence of the rat uricase has been predicted by partial peptide analysis and cDNA cloning [4–6]. Comparison of the sequence with that of soybean uricase showed 40% overall homology with 3 highly homologous regions. Comparison of such evolutionarily separated genes and their products can identify the important regions for the common function and structure, namely, the catalytic domain including substrate binding sites and copper binding sites, the targeting signals to the peroxisome, and the structures forming the peroxisomal core. For these reasons, the function, protein structure, and gene organization relationship of uricase are of particular interest. Furthermore, the genetic background for the loss of uricase in humans and some primates, birds, and amphibians during evolution is also interesting because the distribution of the uricase among species cannot be explained by the phylogenetic tree alone.

In this study, we have isolated the rat chromosomal gene for uricase and sequenced most of the exons and flanking regions. The gene organization is compared with that of the soybean uricase and the evolution of the gene is discussed.

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2. MATERIALS AND METHODS

2.1. *Materials*

Materials were obtained from the following sources: DNA polymerase I, large fragment of DNA polymerase I (Klenow fragment), universal and reverse primers, DNA ligation kit, and restriction enzymes from Takara Shuzo (Kyoto, Japan); [α-^{32}P]dCTP (>700 Ci/mmol) from ICN (Irivine, USA).

2.2. *Screening of rat liver genomic library*

A Sprague-Dawley rat genomic library in Charon 4A (the gift of Dr R.D. Andersen) was screened by plaque hybridization at 42°C in the presence of 50% formamide [7] with ^{32}P-labeled rat uricase cDNA, pP34-3 [4] as a probe. To screen the library with a synthetic 23-mer oligonucleotide complementary to the mRNA region encoding the N-terminal 8 amino acid sequence [4], hydridization was carried out at 42°C in the solution without formamide and the final washing was at 42°C with 2×SSC, 0.1% SDS.

2.3. *DNA sequence analysis*

The DNA fragments containing exons were subcloned into pUC18 and their sequences were directly analyzed by the dideoxy chain termination method following the procedure in [8]. DNA sequence analysis was done using the DNASIS program of Hitachi Software Engineering (Yokohama, Japan).

3. RESULTS AND DISCUSSION

3.1. *Isolation of phage clones carrying a uricase gene*

A rat gene library was screened by plaque hydridization using a ^{32}P-labeled rat uricase cDNA as a probe. Six clones were isolated and two of them were further analyzed after digestion of the cloned DNAs with restriction enzymes, followed by Southern blot hybridization using various fragments of a rat uricase cDNA as probes. Fig. 1 summarizes the restriction maps of the

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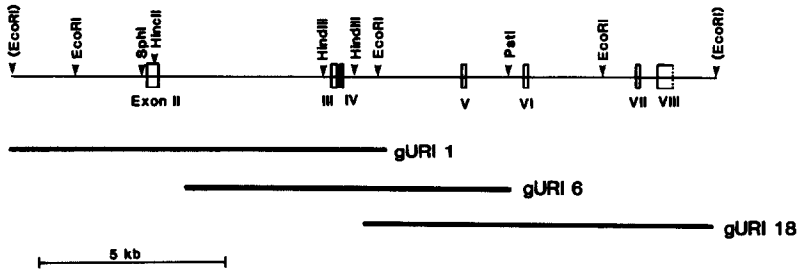

Fig. 1. Restriction maps of the rat uricase gene. Only the relevant restriction sites are shown. Solid boxes indicate exons and lines introns. Regions covered by 3 genomic clones are shown by thick lines.

genomic DNA fragments cloned in gURI1 and gURI18. We did not obtain any evidence from these studies or from a preliminary genomic Southern blot analysis (not shown) to suggest the presence of more than one gene for the uricase in the rat genome.

3.2. *Nucleotide sequence of the rat uricase gene*

The DNA fragments containing exons were subcloned into pUC18 vector and their nucleotide sequences were determined. As shown in Fig. 2, the rat uricase gene spans more than 20 kilobases and the coding region is divided into 8 exons by 7 introns. All the intron/exon junctions obey the AG/GT rule [9].

The exon(s) containing the 5′-noncoding region and the N-terminal coding region has not been cloned and we therefore only tentatively numbered the exons from II to VIII. The end-labeled synthetic oligonucleotide corresponding to the N-terminal region [4] did not hydridize to any fragment of gURI1 (Fig. 1). Furthermore, direct screening of a rat genomic library with the synthetic probe yielded 10 positive clones. However, the rat uricase cDNA probe did not hybridize to any of the phage DNAs and sequencing analyses of the relevant regions of these clones revealed mismatches in every clone ranging from 3 to 5 bases in the stretch of 23 bases. These analyses suggest the existence of a long intron between exon I and exon II and/or the existence of another intron which divides the N-terminal region.

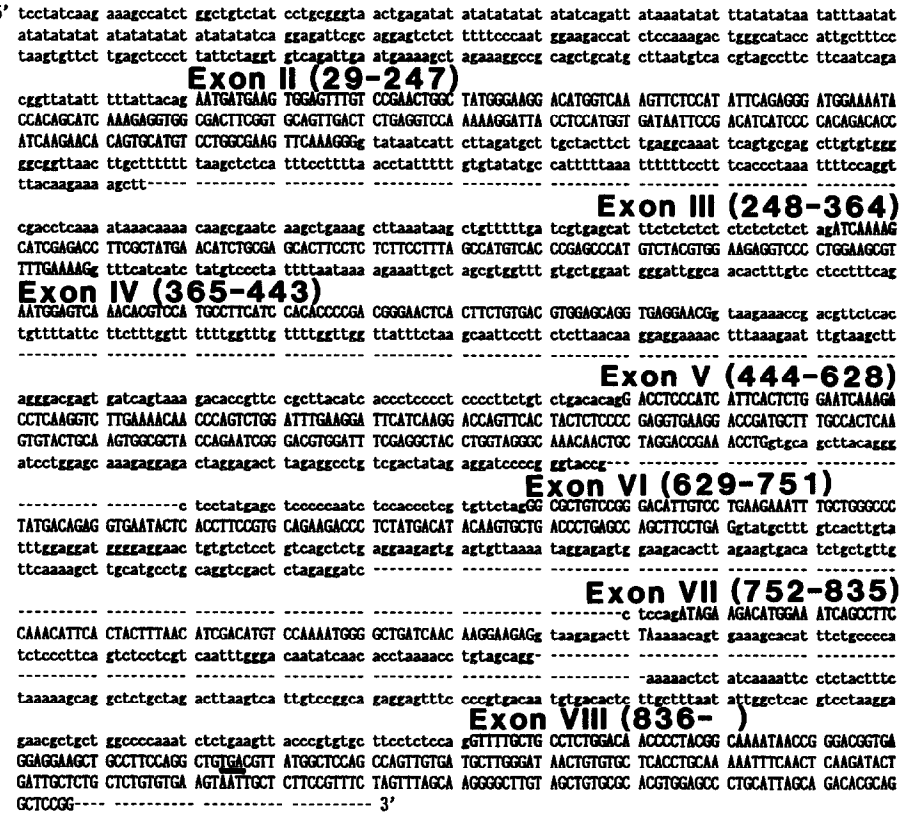

Fig. 2. DNA sequence of the rat uricase gene. Exons and intron sequences are shown in capital and small letters, respectively. The numbers in parentheses refer to the nucleotide numbers of the cDNA [4]. The stop codon TGA is underlined.

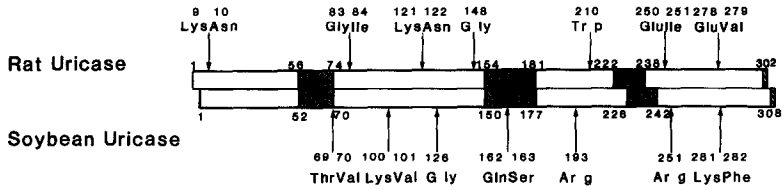

Fig. 3. Comparison of organization of the rat uricase gene with that of the soybean gene. Gene organization is schematically illustrated. The amino acid sequences are aligned with intron positions. Three highly conserved regions are shaded.

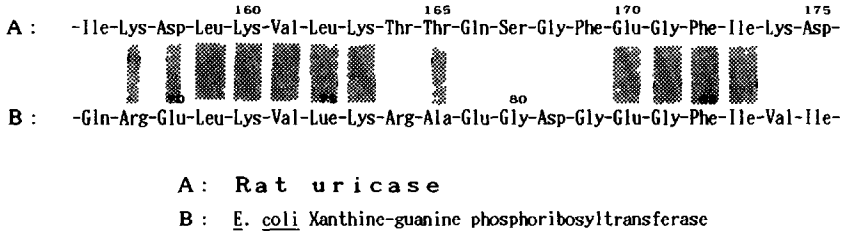

Fig. 4. Comparison of parts of sequences of rat uricase and *E. coli* xanthine-guanine phosphoribosyltransferase [11]. Identical residues in corresponding positions in the two enzymes are fully shaded, and homologous residues are half-shaded.

3.3. *Comparison of the rat uricase gene organization with the soybean gene*

The organization of the rat uricase gene was compared with that of the soybean gene [10] as schematically shown in Fig. 3. They share 3 highly homologous regions [4] and these regions are thought to be important for the enzyme function. The amino acid sequence of the conserved central region of the rat uricase was shown to be similar to that of the *E. coli* xanthine-guanine phosphoribosyltransferase [11], an enzyme in the purine metabolism (Fig. 4). The 3 conserved regions are coded by 3 separate exons in the rat genome, whereas, in the soybean genome, the conserved central region is coded by 2 exons. In addition, only 2 introns out of 7, both of which are at the extreme C-terminal regions, are in similar, but not identical positions and the other 5 are in completely different positions. The large differences in the gene organization between the rat and the soybean uricase gene may have been caused not only by the removal of some ancestral introns but also by the insertion of some introns during their widely separated evolution as suggested in other cases [12].

3.4. *Loss of uricase in humans during evolution*

We have already shown that total loss of uricase in humans is not due to total loss of the gene by cloning and sequencing of a segment of the gene [13]. The segment corresponds to a part of exon VIII of the rat gene (Fig. 3) and neither boundary is conserved as an exon/intron junction. Changes in gene organization may have occurred during evolution before or after introduction of nonsense mutations in the human uricase gene [14].

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