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# Human liver-type arginase gene: structure of the gene and analysis of the promoter region

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ABSTRACT

The gene for human liver-type arginase (EC 3.5.3.1), a urea cycle enzyme, was cloned and the structure was determined. This gene is 11.5 kilobases long and is split into 8 exons. The cap site was determined by nuclease S1 mapping and primer extension. A "TATA box"-like sequence is located 28 bases upstream from the cap site, and a sequence similar to the binding sites of the transcription factor CTF/NF1, a "CAAT box"-binding protein, is located 72 bases upstream. In the 5' end region, sequences resembling the glucocorticoid responsive elements, the cAMP responsive elements, and the enhancer core sequences are present. The immediately 5' flanking region of the human gene up to position -105 is 84% identical with the corresponding segment of the rat gene. In this region of the human gene, one DNase I-protected area and several hypersensitive cleavage sites were detected by footprint analysis, using nuclear extracts from the rat liver. The protected area contains the sequence similar to the binding sites of CTF/NF1 and also overlaps with the sequence resembling the glucocorticoid responsive elements.

## INTRODUCTION

Arginase (EC 3.5.3.1) catalyzes the hydrolysis of arginine to urea and ornithine. Liver-type arginase is localized in the cytosol of the liver cells of ureotelic animals and catalyzes the last step of the urea synthetic pathway. Arginase activity in the rat (1) and human (2,3) liver increases markedly in the perinatal period, in coordination with other urea cycle enzymes. The activities of arginase and of the other urea cycle enzymes are induced also in a coordinated manner by dietary protein (4,5) and hormones such as glucagon and dexamethasone (6,7). These inductions of the urea cycle enzyme activities, in some cases, are associated with increases of mRNA levels for the enzymes (8). A deficiency in human liver arginase results in argininemia, an autosomal recessive disorder accompanied by hyperammonemia, motor difficulties, and mental retardation (9). Arginase isozyme(s) that differs from the liver-type enzyme in catalytic, molecular, and immunological properties, is present in the kidney, small intestine, brain, and lactating mammary gland (10-13). The non-hepatic arginase(s) is assumed to be involved

in the conversion of arginine to proline and glutamate.

As the first step in studying the tissue-specific, developmental, and hormonal regulation of expression of the liver-type arginase gene and in determining the nature of mutation in argininemia, we isolated cDNA clones for the rat (14,15) and human (16) enzyme and determined the entire nucleotide sequences. Sparkes et al. (17) also isolated cDNA clones for human liver-type arginase and assigned the human arginase gene to chromosome band 6q23. We then isolated genomic clones for rat liver-type arginase and determined the entire organization of the gene (18). We now report the isolation and structural analysis of the entire gene for human liver-type arginase and its flanking regions. The gene is 11.5 kilobases (kb) long and is divided into 8 exons. The 5' end region contains several noteworthy sequences that may be important in the regulation of gene expression. The sequences of the 5' and 3' end regions of the human arginase gene were compared with the corresponding sequences of the rat gene, and the highly conserved 5' flanking region was subjected to DNase I footprint analysis.

## MATERIALS AND METHODS

### Isolation and Characterization of Genomic Clones

A human genomic library was prepared by ligation of a partial Sau3AI digest of peripheral blood DNA and a BamHI digest of bacteriophage EMBL4, as described (19). The library was screened with nick-translated fragments excised from the human liver arginase cDNA clones phARG6 and phARG109 (16). Phage DNAs of positive clones were characterized by restriction mapping and by Southern blot hybridization analysis (20). Subclones were constructed with plasmid pUC18 as a vector, and the nucleotide sequences of the double-stranded plasmid subclones were determined by the dideoxynucleotide chain-termination method (21,22), using synthetic oligonucleotide primers complementary to the sequences of the vector and the arginase gene. Oligonucleotide primers were synthesized by a DNA synthesizer (Model 381A, Applied Biosystems, Foster City).

### Analysis of the 5' End of Arginase mRNA

Poly(A)$^+$RNA was prepared from an autopsy liver sample by guanidium thiocyanate-phenol extraction (23) followed by oligo(dT)-cellulose chromatography. Determination of the 5' end of the arginase mRNA by nuclease S1 mapping (24) and primer extension (25) was performed as described. The probe for the S1 mapping was prepared as follows. Since no restriction site adequate for preparation of the probe was found in the first exon of the human

arginase gene, the arginase cDNA was fused to the cloned 5' end region of the gene at the HaeII site (position +64) in the first exon in order to utilize a restriction site in the cDNA portion. Namely, both the EcoRI (~-3.9 k)-HaeII (+64) fragment of the genomic DNA and the HaeII (+64)-BamHI (~+1.1 k, situated in the multiple cloning site of pUC9) fragment of cDNA clone phARG112 (26) were inserted into EcoRI/BamHI-cut pUC19. The PvuII (-66)-HaeIII (+138, situated in the cDNA portion) fragment $^{32}$P-labeled at the 5' end of the HaeIII site was prepared from the resultant fusion gene and used as the probe.

DNase I Footprint Analysis

Nuclear extracts from the rat liver were prepared as described by Gorski et al. (27). Binding reaction was carried out for 15 min on ice in 50 μl of the mixture containing 25 mM Tris-HCl (pH 7.6), 50 mM KCl, 6 mM $MgCl_2$, 0.1 mM EDTA, 0.5 mM dithiothreitol, 12% glycerol, 0.3 μg of poly(dI-dC), 10 fmol of the 5' end-labeled DNA fragment (about 1 X $10^5$ dpm), and up to 135 μg of protein of the nuclear extracts. After supplying 50 μl of the solution containing 5 mM $CaCl_2$ and 10 mM $MgCl_2$ to the mixture, freshly diluted DNase I from bovine pancreas (DPRF, Cooper Biomedical Inc., Malvern) of an empirically adjusted amount was added, and digestion was allowed to proceed for 60 s at room temperature. The reaction was stopped by the addition of 100 μl of 1% sodium dodecyl sulphate containing 20 mM EDTA and 200 mM NaCl. After the addition of 10 μg of calf thymus tRNA as a carrier, nucleic acids were extracted three times with phenol-chloroform, and collected with ethanol precipitation. Electrophoresis was carried out in a 6% acrylamide-7 M urea gel.

## RESULTS AND DISCUSSION

### Isolation and Characterization of the Human Liver-type Arginase Gene

A phage library constructed from human peripheral blood was screened for the arginase gene. 14 independent clones were isolated and analyzed by restriction enzyme digestion (Fig. 1). These clones overlapped and spanned about 40 kb.

To define positions and boundaries of the exon blocks, the restriction fragments identified by Southern hybridization were subcloned and their sequences were determined. The results are shown in Figs. 1 and 2. The gene is 11.5 kb long and is divided into 8 exons. The 8 exons range in size from 73 bases (exon 2) to 587 bases (exon 8), and the 7 introns range from 186 bases (intron 6) to 3.6 kb (intron 1). The exon-intron organization of the human arginase gene is similar to that of the rat gene (18) except for sizes of

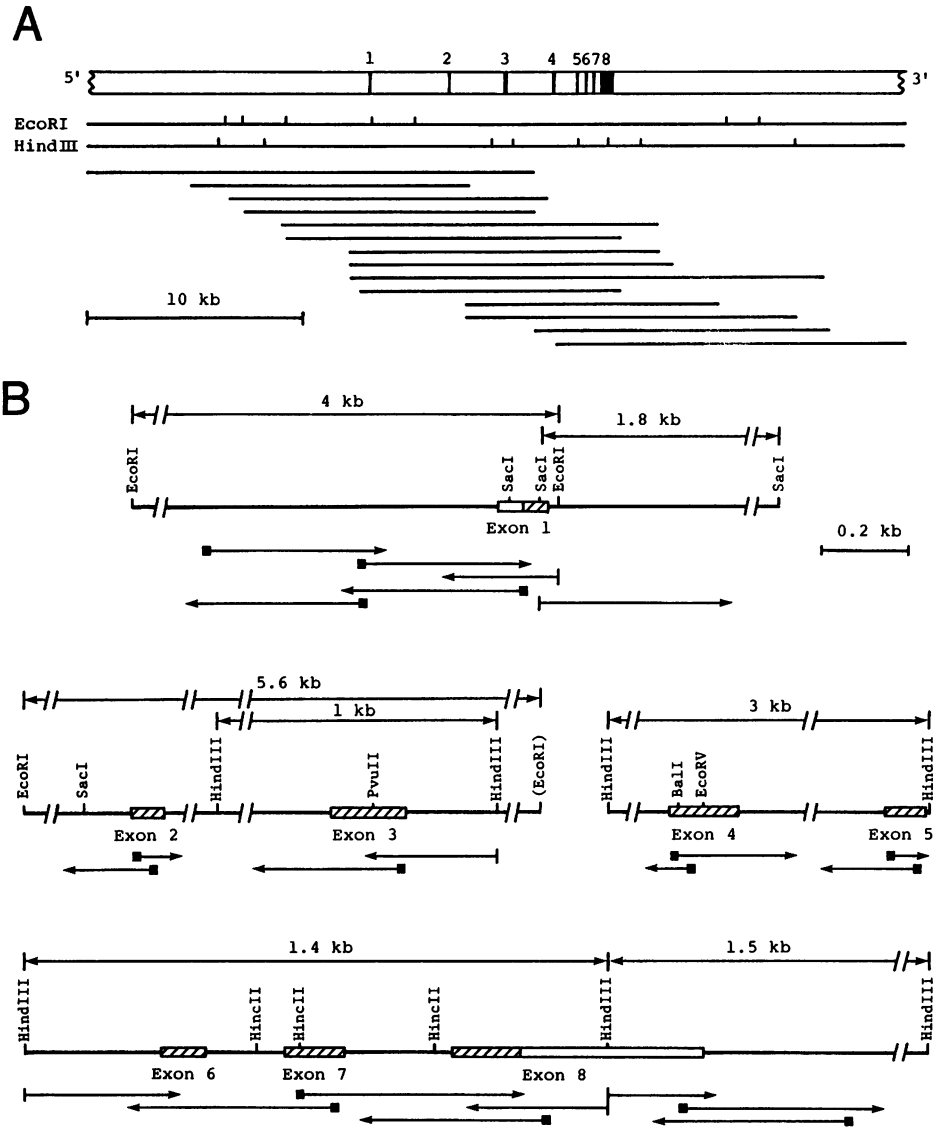



**Figure 1. A physical map of the human liver-type arginase gene (A) and the sequencing strategy (B).** (A) The structure of the gene is shown as the bar at the top of the diagram. Exons 1-8 are the filled areas. EcoRI and HindIII sites are shown below the gene structure. The genomic DNA fragments contained in the phage clones are shown below. (B) Subcloned regions are shown by bidirectional arrows at the top. Only relevant restriction sites are indicated. An EcoRI site parenthesized derives from the multiple cloning sites of EMBL4. Positions of exons determined are shown by boxes in the thick lines at the center. Open areas in the boxes indicate 5' and 3' non-coding regions,

and hatched areas coding regions. The horizontal arrows at the bottom indicate the direction and extent of sequence determination starting from the universal or reverse primer complementary to portions of pUC18, shown by short vertical lines, or from the specific primers (17-mer), shown by filled boxes.

introns 1 and 2. Those of the human gene are 3.6 and 2.5 kb, respectively, whereas those of the rat gene are 4.3 kb and 1.9 kb, respectively. All of the splice donor and acceptor sites conform to the GT/AG rule (28) for nucleotides immediately flanking exon borders. The locations of the seven introns in the mRNA sequence are completely conserved between the human and rat (18) genes.

We previously determined the sequences of several cDNA clones for human arginase and found several nucleotide substitutions in the protein coding region (26). Relevant nucleotides of the gene sequence determined here are identical with the most frequent alternatives among these substitutions.

Characterization of the 5' and 3' Ends of the Gene

DNA sequence around the 5' end of the human liver-type arginase gene is shown in Fig. 3A. The cap site was determined by nuclease S1 mapping and primer extension using human liver poly(A)$^+$RNA. In order to obtain an S1 mapping probe which is long enough to anneal efficiently with the arginase mRNA, genomic DNA containing 5' end region was fused with cDNA at a restriction site situated in the first exon, and the PvuII (-66)-HaeIII (+138 in the cDNA region) fragment was excised from the fused gene. S1 mapping analysis with this probe gave protected fragments of 139, 138, 136, 135, and 134 bases long (Fig. 4A). In primer extension analysis, reverse transcripts of 99 and 96 bases long were synthesized, starting from the primer labeled at position +99 (Fig. 4B). These two transcripts corresponded to the S1 products of 138 and 135 bases long, respectively. These results indicate that there are two distinct 5' ends for the human arginase mRNA situated three bases apart. These sites correspond to nucleotides 58 and 55 bases upstream from the initiation codon. The more upstream situated 5' end is numbered +1. The 5' untranslated region of the human gene (58 nucleotides) is shorter than that of the rat gene (93 nucleotides) (see Fig. 6A). The situation of the more upstream situated cap site of the human arginase gene is essentially identical with that of the rat gene (see also Fig. 7B). The cap site is overlapped by a sequence of dyad symmetry (DS-5), which might serve as a recognition site for the DNA-binding protein(s) (29).

The sequence TATAA (-28 to -24) identical with the canonical "TATA box" is situated at the ordinary location and is presumably functional. The sequence AGCCAG resembling the consensus sequence AGCCAA (30) for the binding sites of a transcription factor CTF/NF1 (30,31), a "CAAT box"-recognizing

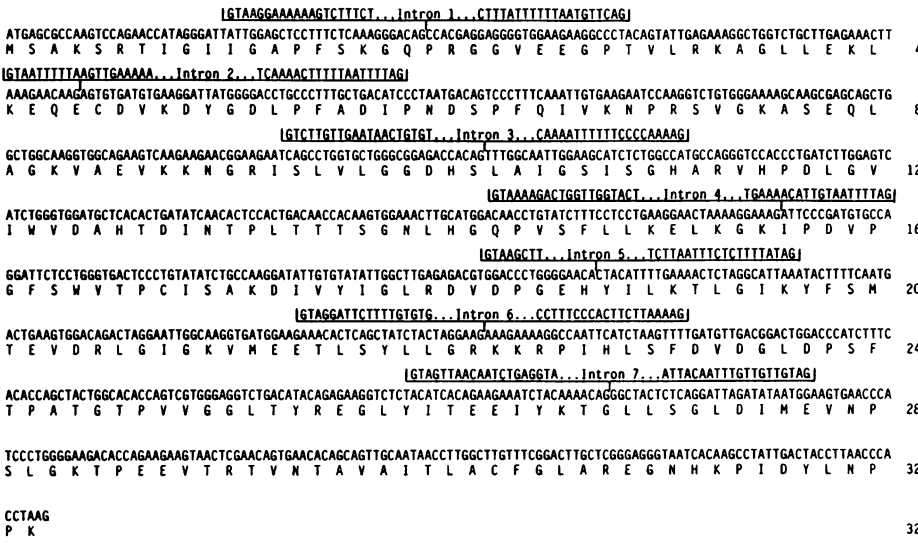



**Figure 2. The exon-intron organization of the human liver-type arginase gene.** The locations of the seven introns are indicated above the contiguous protein-coding sequence. 5' and 3' untranslated sequences are not included.

protein, is present at positions -72 to -67. A sequence GAGGAAAG similar to the viral and cellular enhancer core sequences (32), is found at positions -366 to -359 (Fig. 5A). Sequences resembling the glucocorticoid responsive elements (33) are situated at positions -319 to -333 (antisense strand), -225 to -239 (antisense strand), and -74 to -60 (Fig. 5B). The two sequences most upstream are overlapped by sequences of dyad symmetry (DS-1 and DS-2). The most downstream sequence is overlapped by the putative "CAAT box". These sequences might be involved in induction of liver-type arginase by glucocorticoid (6-8). A sequence TGAAGTCA similar to the cAMP responsive elements (34) is situated at positions -289 to -282 (Fig. 5C), and might be involved in induction of this enzyme by dibutyryl cAMP (8) and glucagon (6,7). The region corresponding to positions -191 to -117 contains three long direct repeats (DR-4, the third repeat is shorter), and the most upstream situated two sets of the repeats contain the sequences of dyad symmetry (DS-3 and DS-4). There are several other sets of direct repeats in the 5' end region (DR-1 to DR-3).

The DNA sequence around the 3' end region of the arginase gene is shown in Fig. 3B. The 3' untranslated region contains 423 nucleotides. A poly(A)-

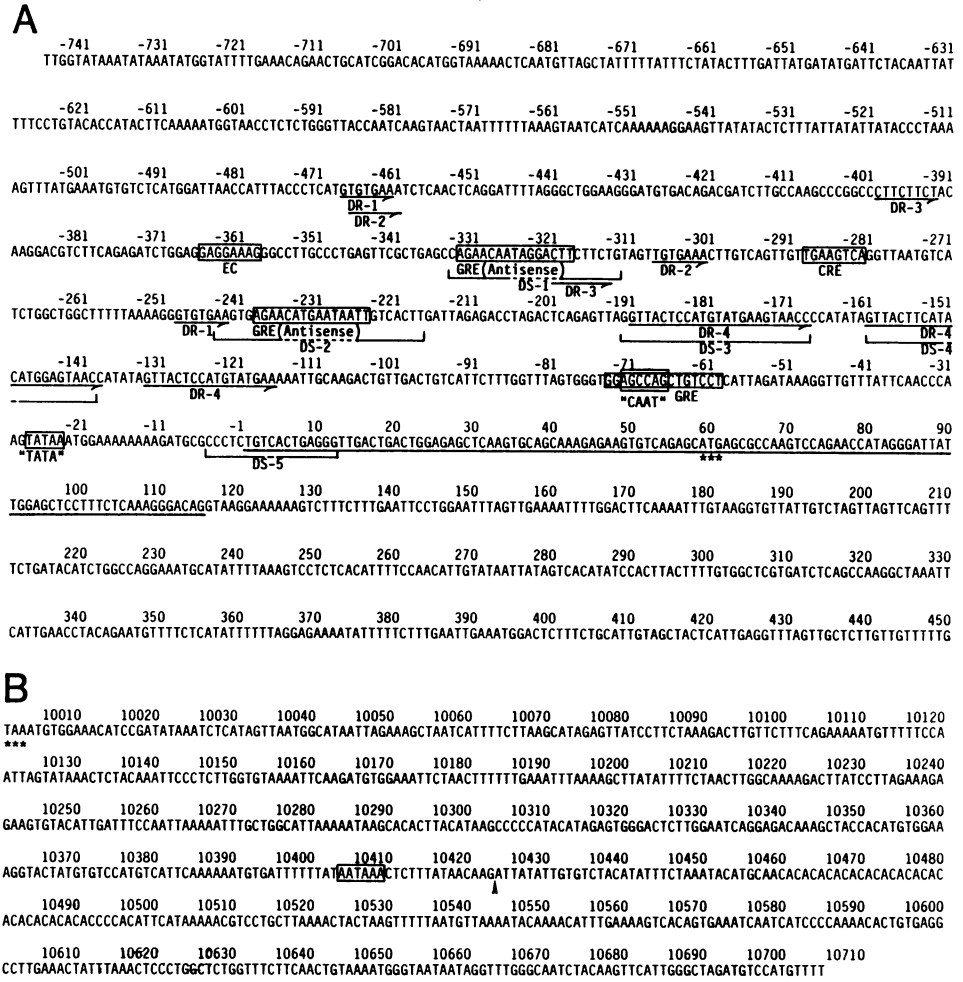



**Figure 3. Nucleotide sequences of the 5' (A) and 3' (B) end regions.** (A) The underline (+1 to +115) shows the first exon. The initiation codon ATG is marked with asterisks. The boxed areas with the symbols show the sequences resembling the followings; "TATA", "TATA box"; "CAAT", the consensus sequence for binding sites of CTF/NF1 (30,31), a "CAAT box"-binding protein; EC, enhancer core sequences (32); GRE, the consensus sequence for glucocorticoid responsive elements (33); CRE, the consensus sequence for cAMP responsive elements (34). Brackets with the intervening broken lines, marked with DS-1 to DS-5, indicate the sequences of dyad symmetry. Paired arrows DR-1 to DR-4 indicate direct repeats. (B) The sequence downstream of the termination codon TAA (marked with asterisks) is shown. The nucleotide corresponding to the termination codon is numbered 10,001. The poly(A)-addition site is shown by the arrowhead, and the putative poly(A)-addition signal is boxed.

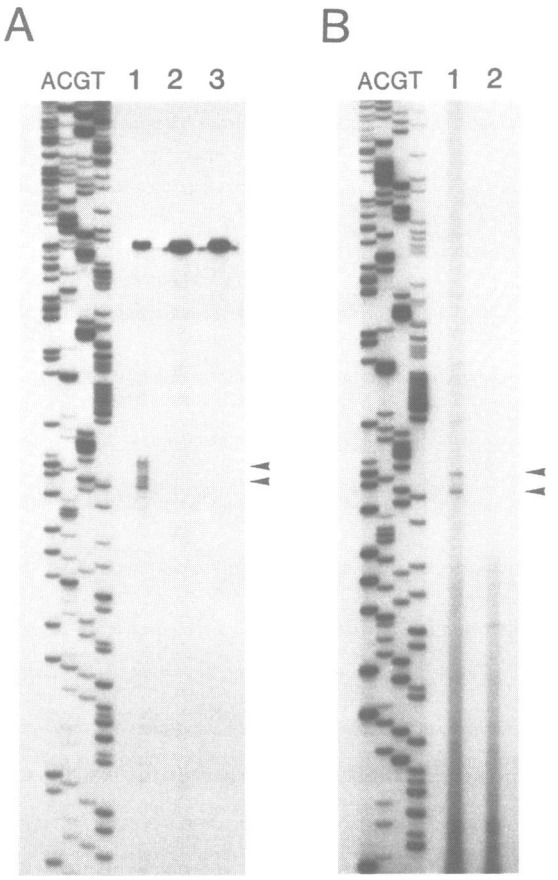



**Figure 4. Determination of the 5' end of human liver-type arginase mRNA by nuclease S1 mapping (A) and primer extension (B).** Poly(A)$^+$RNA from human autopsy liver was analyzed (lanes 1 of A and B). (A) The probe was the PvuII (-66)-HaeIII (+138 situated in the cDNA portion, see MATERIALS AND METHODS) fragment $^{32}$P-labeled at the 5' end of the HaeIII site with [$\gamma$-$^{32}$P]ATP and T4 polynucleotide kinase. Hybridization was carried out with 10 fmol of the probe (about 1 X $10^5$ dpm) and 3 $\mu$g of the poly(A)$^+$RNA at 50 °C for 20 h, and the mixture was subjected to nuclease S1 digestion. Lane 3 shows the S1 probe. (B) The primer was a synthetic oligonucleotide (25-mer, complementary to positions +75 to +99) $^{32}$P-labeled at the 5' end with [$\gamma$-$^{32}$P]ATP and T4 polynucleotide kinase. Annealing was carried out with 0.35 pmol of the primer (3 X $10^6$ dpm) and 3 $\mu$g of the poly(A)$^+$RNA, and the mixture was subjected to reverse transcription. In experiments of lanes 2 of (A) and (B), calf liver tRNA was used as a control. To compare the products directly with the genomic nucleotide sequence, the 5' ends of the probe and of the primer were arranged to coincide with the 5' ends of primers of the dideoxynucleotide sequencing (lanes A, C, G, and T). Portions of samples were electrophoresed in a 6% acrylamide-7 M urea gel. Arrowheads indicate the positions assigned to the 5' ends of the mRNA.

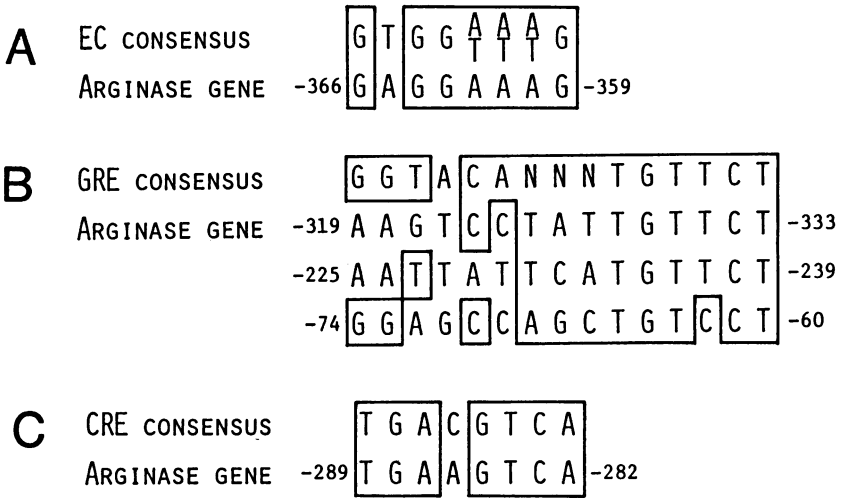



**Figure 5. Similarities between the 5' end region sequences of the human liver-type arginase gene and the consensus sequence for the enhancer core sequences (EC consensus, Ref. 32) (A), that for the glucocorticoid responsive elements (GRE consensus, Ref. 33) (B), and that for the cAMP responsive elements (CRE consensus, Ref. 34) (C).** Sequences of the arginase gene at positions -319 to -333, and -225 to -239 are on the antisense strand. Matched nucleotides are boxed.

addition signal AATAAA is located 14 bases upstream from the poly(A) tail.

Sequence Comparison of the 5' and 3' End Regions of the Human and Rat Genes

Sequence of the 5' end region of the human arginase gene was compared with that of the rat arginase gene (18), by a dot matrix analysis (Fig. 6A). There are several highly conserved segments in the 5' flanking regions between the human and rat genes. The immediately 5' flanking sequence of the human gene up to position -105, including the putative "TATA box" and "CAAT box", is 84% identical with the corresponding portion of the rat gene, without counting several small gaps. The long direct repeats situated at positions -191 to -117 (DR-4 in Fig. 3A) in the human gene were missing in the rat gene, and these repeats were apparently inserted or deleted after separation of the two species. This region may play a role in regulation of gene expression specific to the human gene. Alternatively, this region might simply have arisen by insertion of an unrelated sequence.

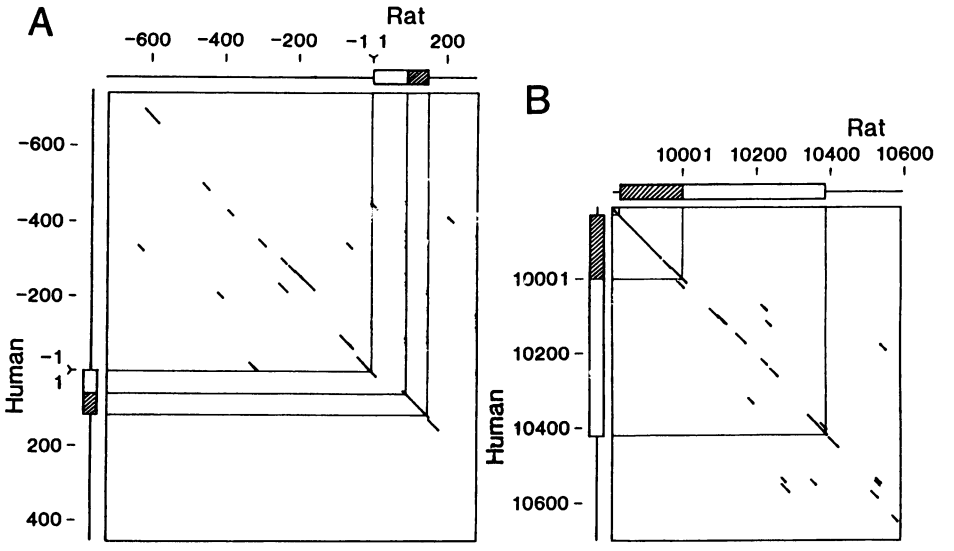


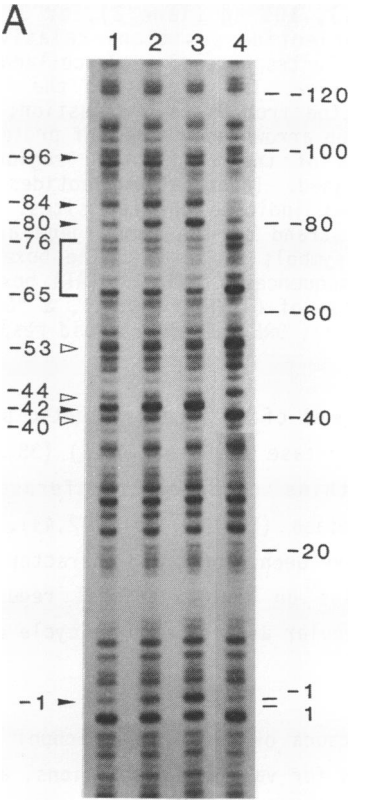

**Figure 6. Dot matrix comparison of the 5' (A) and 3' (B) end region sequences of the human and rat liver-type arginase genes.** The human sequences are from Fig. 3 and the rat sequences from Ref. 18. A computer program was used to generate diagonal dots at the range of 15 nucleotides in which 10 nucleotides or more coincide. The open and hatched boxes beside the matrices indicate the untranslated and translated regions of the first exons (A) and the last exons (B) of the arginase genes.

Figure 6B shows sequence comparison of the 3' end regions of the human and rat arginase genes. The 3' untranslated sequences are rather well conserved between the human and rat genes along the almost entire sequences (61% identical without counting the gaps). There are several highly conserved segments in the 3' flanking regions.

DNase I Footprint Analysis of the 5' Flanking Region

In order to detect protein factors which interact with the arginase promoter sequences, DNase I footprint analysis was carried out with nuclear extracts from the rat liver (Fig. 7). The extracts were prepared by the procedure of Gorski et al. (27). The probe used was the coding strand of the human gene, 5' end-labeled at position +62, and this probe spans over the highly conserved portion of the immediately 5' flanking region (up to position -105 of the human gene, see Figs. 6A and 7B). A protected region was detected at positions -65 to -76. In addition, hypersensitive cleavage sites were found

at positions -1, -42, -80, -84, and -96, and protected sites at positions -40, -44, and -53. The protected region at positions -65 to -76 is situated in a block of sequences highly conserved between the human and rat genes (positions -64 to -81 of the human gene). This region contains the sequence that resembles the consensus sequence for the binding sites of CTF/NF1 (30,31), a "CAAT box"-recognizing protein. Recently it was shown that CTF/NF1-related proteins are relatively abundant in liver nuclei, and may play a role in liver-specific expression of the serum albumin gene (35,36). In the human arginase gene, the protected region overlaps with the sequence similar to the glucocorticoid responsive elements. On the other hand, the corresponding region of the rat gene overlaps with the sequence similar to the binding sites of the transcription factor Sp1 (37). It remains to be elucidated whether or not a factor(s) that binds to the protected region is identical with these previously characterized transcription factors, and what is the function of the binding factor(s).



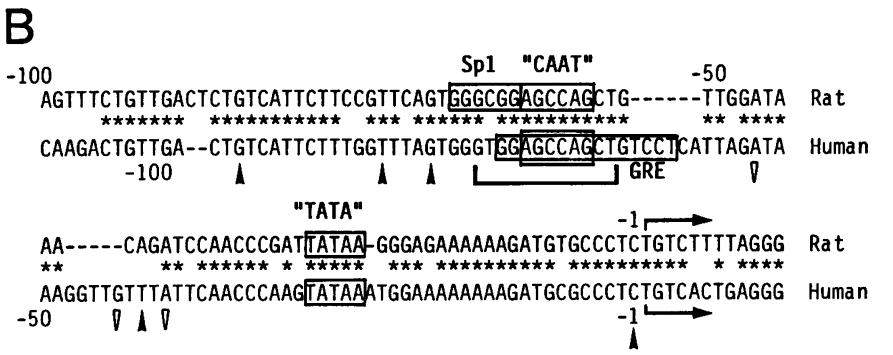



**Figure 7. Footprint analysis of the 5' end region of human liver-type arginase gene with rat liver nuclear extracts.** (A) The probe was the BglII (-371)-HaeII (+62) fragment $^{32}$P-labeled at the 5' end (+62) of the coding strand. Binding reaction was carried out with 0 μg (lane 4), 15 μg (lane 1), 45 μg (lane 2), or 135 μg protein (lane 3) of rat liver nuclear extracts. DNase I digestion was performed with 2 ng (the lower half of lane 4), 5 ng (the upper half of lane 4), 35 ng (lane 1), 105 ng (lane 2), or 315 ng (lane 3) of bovine pancreatic DNase I. Nucleotide positions relative to the cap site were assigned referring to coelectrophoresed sequence ladders (not shown), and were indicated on the right side. The symbols on the left side are as follows; bracket, a region protected from DNase I digestion; filled arrowheads, sites of enhanced cleavage; open arrowheads, sites of protected cleavage.
(B) Nucleotide sequences of the 5' flanking regions of the rat and human arginase genes were aligned. Identical nucleotides are shown by asterisks. Beginning sites of arrows indicate the cap sites. Results of the footprint analysis on the coding strand of the human gene are shown below the human sequence with the same symbols as in (A). The boxed areas with the symbols resemble the following sequences; "TATA", "TATA box"; "CAAT", the consensus sequence for binding sites of CTF/NF1 (30,31), a "CAAT box"-binding protein; Sp1, Sp1-binding sites (37); GRE, glucocorticoid responsive elements (33).

Among the five enzymes of the urea synthetic pathway, the genes for rat carbamoyl phosphate synthetase I (EC 6.3.4.16) (38,39), rat (19), mouse (40) and human (41) ornithine carbamoyltransferase (EC 2.1.3.3), human argininosuccinate synthetase (EC 6.3.4.5) (42,43), and rat (18) and human (this paper) arginase have been cloned and characterized. These genomic clones should facilitate studies on transcriptional regulation of the urea cycle enzyme genes and on molecular aspects of urea cycle enzyme disorders.

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REFERENCES

1. Lamers, W. H., Mooren, P. G., De Graaf, A. and Charles, R. (1985) Eur. J. Biochem. **146**, 475-480.
2. Kennan, A. L. and Cohen, P. P. (1961) Proc. Soc. Exp. Biol. Med. **106**, 170-173.
3. Guha, S. K. and Mukherjee, K. L. (1974) Biochim. Biophys. Acta **372**, 285-290.
4. Schimke, R. T. (1962) J. Biol. Chem. **237**, 459-468.
5. Mori, M., Miura, S., Tatibana, M. and Cohen, P. P. (1981) J. Biol. Chem. **256**, 4127-4132.
6. Lin, R. C., Snodgrass, P. J. and Rabier, D. (1982) J. Biol. Chem. **257**, 5061-5067.
7. Gebhardt, R. and Mecke, D. (1979) Eur. J. Biochem. **97**, 29-35.
8. Morris, S. M., Jr., Moncman, C. L., Rand, K. D., Dizikes, G. J., Cederbaum, S. D. and O'Brien, W. E. (1987) Arch. Biochem. Biophys. **256**, 343-353.
9. Walser, M. (1983) In Stanbury, J. B., Wyngaarden, J. B., Fredrickson, D. S., Goldstein, J. L. and Brown, M. S. (eds) The Metabolic Basis of Inherited Disease 5th edn, McGraw- Hill, New York, pp. 402-438.
10. Glass, R. D. and Knox, W. E. (1973) J. Biol. Chem. **248**, 5785-5789.
11. Herzfeld, A. and Raper, S. M. (1976) Biochem. J. **153**, 469-478.
12. Skrzypek-Osiecka, I., Robin, Y. and Porembska, Z. (1983) Acta Biochim. Pol. **30**, 83-92.
13. Spector, E. B., Rice, S. C. H. and Cederbaum, S. D. (1983) Pediatr. Res. **17**, 941-944.
14. Kawamoto, S., Amaya, Y., Oda, T., Kuzumi, T., Saheki, T., Kimura, S. and Mori, M. (1986) Biochem. Biophys. Res. Commun. **136**, 955-961.
15. Kawamoto, S., Amaya, Y., Murakami, K., Tokunaga, F., Iwanaga, S., Kobayashi, K., Saheki, T., Kimura, S. and Mori, M. (1987) J. Biol. Chem. **262**, 6280-6283.
16. Haraguchi, Y., Takiguchi, M., Amaya, Y., Kawamoto, S., Matsuda, I. and Mori, M. (1987) Proc. Natl. Acad. Sci. USA **84**, 412-415.
17. Sparkes, R. S., Dizikes, G. J., Klisak, I., Grody, W. W., Mohandas, T., Heinzmann, C., Zollman, S., Lusis, A. J. and Cederbaum, S. D. (1986) Am. J. Hum. Genet. **39**, 186-193.
18. Ohtake, A., Takiguchi, M., Shigeto, Y., Amaya, Y., Kawamoto, S. and Mori, M. (1988) J. Biol. Chem. **263**, 2245-2249.
19. Takiguchi, M., Murakami, T., Miura, S. and Mori, M. (1987) Proc. Natl. Acad. Sci. USA **84**, 6136-6140.
20. Southern, E. M. (1975) J. Mol. Biol. **98**, 503-517.
21. Sanger, F., Nicklen, S. and Coulson, A. R. (1977) Proc. Natl. Acad. Sci. USA **74**, 5463-5467.
22. Hattori, M. and Sakaki, Y. (1986) Anal. Biochem. **152**, 232-238.
23. Chomczynski, P. and Sacchi, N. (1987) Anal. Biochem. **162**, 156-159.
24. Berk, A. J. and Sharp, P. A. (1977) Cell **12**, 721-732.
25. Agarwal, K. L., Brunstedt, J. and Noyes, B. E. (1981) J. Biol. Chem. **256**, 1023-1028.

26. Haraguchi, Y., Takiguchi, M., Matsuda, I. and Mori, M. (1988) Jpn. J. Human Genet., in press.
27. Gorski, K., Carneiro, M. and Schibler, U. (1986) Cell **47**, 767-776.
28. Breathnach, R. and Chambon, P. (1981) Annu. Rev. Biochem. **50**, 349-383.
29. Pabo, C. O. and Sauer, R. T. (1984) Annu. Rev. Biochem. **53**, 293-321.
30. Jones, K. A., Kadonaga, J. T., Rosenfeld, P. J., Kelly, T. J. and Tjian, R. (1987) Cell **48**, 79-89.
31. Rosenfeld, P. J. and Kelly, T. J. (1986) J. Biol. Chem. **261**, 1398-1408.
32. Weiher, H., König, M. and Gruss, P. (1983) Science **219**, 626-631.
33. Jantzen. H.-M., Strähle, U., Gloss, B., Stewart, F., Schmid, W., Boshart, M., Miksicek, R. and Schütz, G. (1987) Cell **49**, 29-38.
34. Montminy, M. R., Sevarino, K. A., Wagner, J. A., Mandel, G. and Goodman, R. H. (1986) Proc. Natl. Acad. Sci. USA **83**, 6682-6686.
35. Cereghini, S., Raymondjean, M., Carranca, A. G., Herbomel, P. and Yaniv, M. (1987) Cell **50**, 627-638.
36. Lichtsteiner, S., Wuarin, J. and Schibler, U. (1987) Cell **51**, 963-973.
37. Kadonaga, J. T., Jones, K. A. and Tjian, R. (1986) Trends Biochem. Sci. **11**, 20-23.
38. Nyunoya, H., Broglie, K. E., Widgren, E. E. and Lusty, C. J. (1985) J. Biol. Chem. **260**, 9346-9356.
39. Lagacé, M., Howell, B. W., Burak, R., Lusty, C. J. and Shore, G. C. (1987) J. Biol. Chem. **262**, 10415-10418.
40. Scherer, S. E., Veres, G. and Caskey, C. T. (1988) Nucleic Acids Res. **16**, 1593-1601.
41. Hata, A., Tsuzuki, T., Shimada, K., Takiguchi, M., Mori, M. and Matsuda, I. (1988) J. Biochem. (Tokyo) **103**, 302-308.
42. Freytag, S. O., Beaudet, A. L., Bock, H. G. O. and O'Brien, W. E. (1984) Mol. Cell. Biol. **4**, 1978-1984.
43. Jinno, Y., Matuo, S., Nomiyama, H., Shimada, K and Matsuda, I. (1985) J. Biochem. (Tokyo) **98**, 1395-1403.