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# Human type II arginase: sequence analysis and tissue-specific expression

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**Abstract**

A full-length cDNA encoding type II arginase was isolated from a human kidney cDNA library and its sequence compared to those of vertebrate type I arginases as well as to arginases of bacteria, fungi and plants. The predicted sequence of human type II arginase is 58% identical to the sequence of human type I arginase but is 71% identical to the sequence of *Xenopus* type II arginase, suggesting that duplication of the arginase gene occurred before mammals and amphibians diverged. Seven residues known to be essential for activity were found to be conserved in all arginases. Type II arginase mRNA was detected in virtually all human and mouse RNA samples tested whereas type I arginase mRNA was found only in liver. At least five mRNA species hybridizing to type II arginase cDNA were found in the human RNA samples whereas only a single type II arginase mRNA species was found in the mouse. This raises the possibility that the multiple type II arginase mRNAs in humans arise from differential RNA processing or usage of alternative promoters. © 1997 Elsevier Science B.V.

*Keywords:* Kidney; Mouse; Nitric oxide; Ornithine; Xenopus

## 1. Introduction

At least two isoforms of mammalian arginase (EC 3.5.3.1) exist, both of which catalyze the hydrolysis of arginine to ornithine and urea (reviewed in Jenkinson et al., 1996). However, these isoforms (types I and II) differ in tissue distribution, immunologic reactivity, physiologic function and certain enzymatic properties (e.g., Herzfeld and Raper, 1976; Spector et al., 1983; Jenkinson et al., 1996). Moreover, the two arginase isoforms are localized in different subcellular compartments, with the type I isoform located in the cytosol and the type II isoform located in mitochondria (Jenkinson et al., 1996). The physiologic function of type I arginase, which is highly expressed in liver as a component of the urea cycle, has been known for much of this century. In contrast, the physiologic role(s) of type II arginase is still poorly understood. Because the substrate arginine as well as the products ornithine and urea are each involved in a variety of distinct physiologic processes, the function of type II arginase expression very likely varies among different tissues. For example, type II arginase may play a role in regulating the synthesis of nitric oxide, proline, glutamate and polyamines (for reviews, see Jenkinson et al., 1996; Morris, 1996). Thus, the availability of a cloned type II arginase cDNA would provide a powerful tool for investigating the structure and physiologic functions of this enzyme in mammals.

The only sequences reported for mammalian arginases are those of the type I isoform (Haraguchi et al., 1987; Kawamoto et al., 1987). Mammalian type I arginases are sufficiently different from type II arginases that they are not immunologically cross-reactive (Herzfeld and Raper, 1976; Spector et al., 1983, 1994) and attempts to achieve cross-hybridization using the type I arginase cDNA at reduced stringencies have not been successful (unpublished results). Recently, however, the first cDNA clones encoding type II arginase were isolated from *Xenopus laevis* (Patterson and Shi, 1994). Preliminary experiments in this laboratory showed that the *Xenopus* cDNA encoding type II arginase cross-hybridized with an apparently homologous mRNA in rat kidney RNA, indicating that the *Xenopus* cDNA could be used to identity a mammalian homolog. Thus, the objectives of this study were to isolate and characterize a human type II arginase cDNA, to determine its relationship to type I arginases by sequence comparison, and to compare the expression of type I and II arginases in human and mouse tissues.

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Abbreviations: bp, base pair(s); kb, kilobase(s) or 1000 bp.


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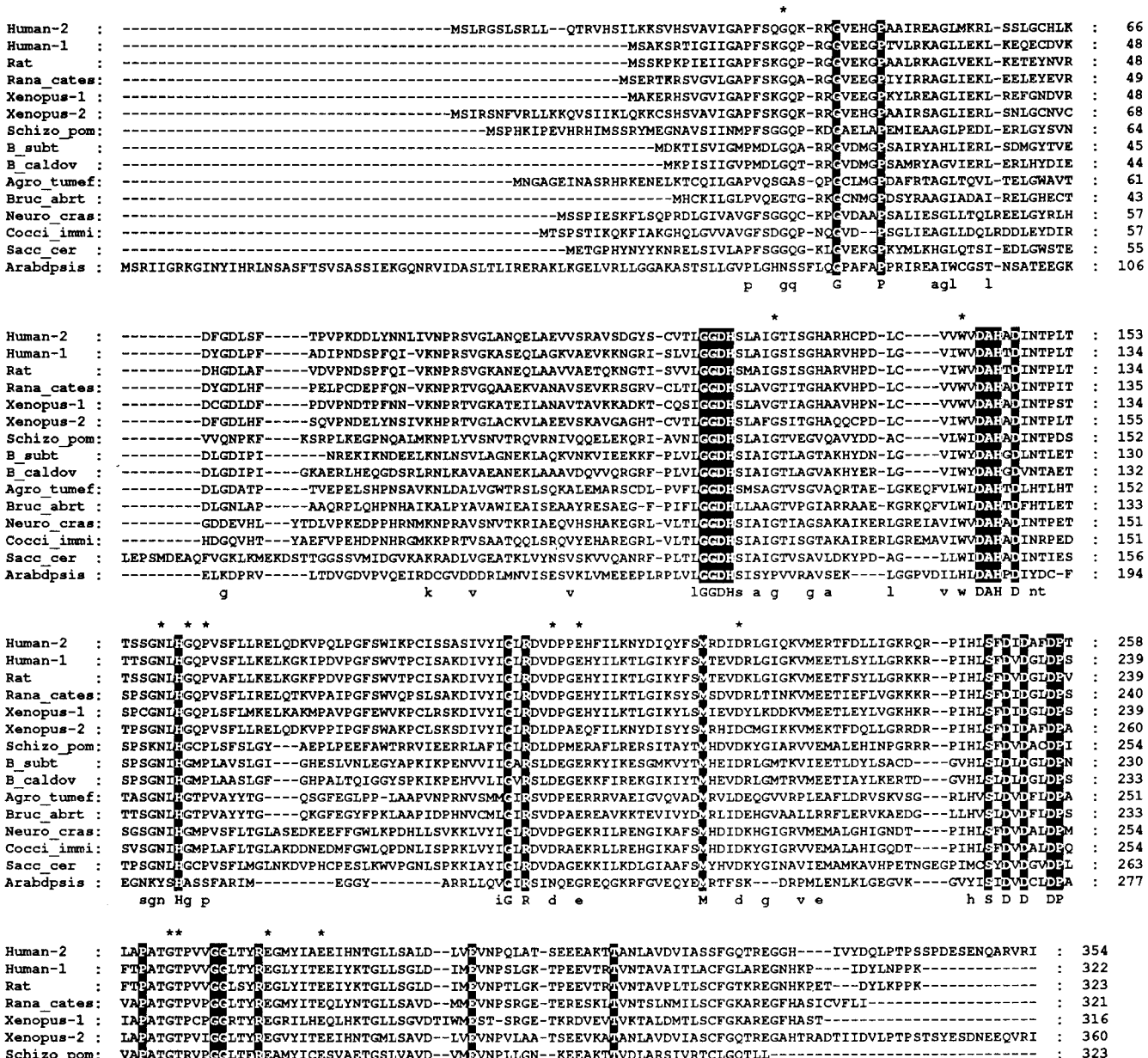


Fig. 1. Amino acid sequence of human type II arginase and comparison with other arginases. Letters on black background and capital letters below alignment represent residues conserved in all arginases; lower case letters below alignment represent residues conserved in at least 12 of 15 arginases; asterisks above alignments identify residues conserved in all species except for *Arabidopsis*. **Methods**: A full-length type II arginase cDNA clone was isolated by screening a SUPERSCRIPT human kidney cDNA library (Life Technologies, Inc.) with $^{32}$P-labeled arg-2 cDNA of *Xenopus laevis* (Patterson and Shi, 1994). Filter hybridizations were carried out using the conditions of Amasino (1986), except that reduced-stringency hybridizations and post-hybridization washes were carried out at 37°C and 43°C, respectively. Both strands of cloned cDNA were sequenced on an ABI PRISM Model 377 DNA Sequencer according to the manufacturer's protocols. The cDNA sequence for human type II arginase has been deposited in GenBank with accession No. U82256. Protein sequences of multiple arginases were initially aligned using the program CLUSTAL W (Thompson et al., 1994); alignments in conserved regions were optimized by using the program SAGA (Notredame and Higgins, 1996). Using the recently published crystal structure of rat type I arginase (Kanyo et al., 1996) as a guide, the alignment was finally adjusted manually in order to obtain an optimal alignment. Final alignment format was prepared by Genedoc (http://www.cris.com/~Ketchup/genedoc.shtml). Published arginase sequences used for comparison were from *Xenopus laevis* (type II; clone arg-2; Patterson and Shi, 1994), *Xenopus laevis* (type I; Xu et al., 1993),

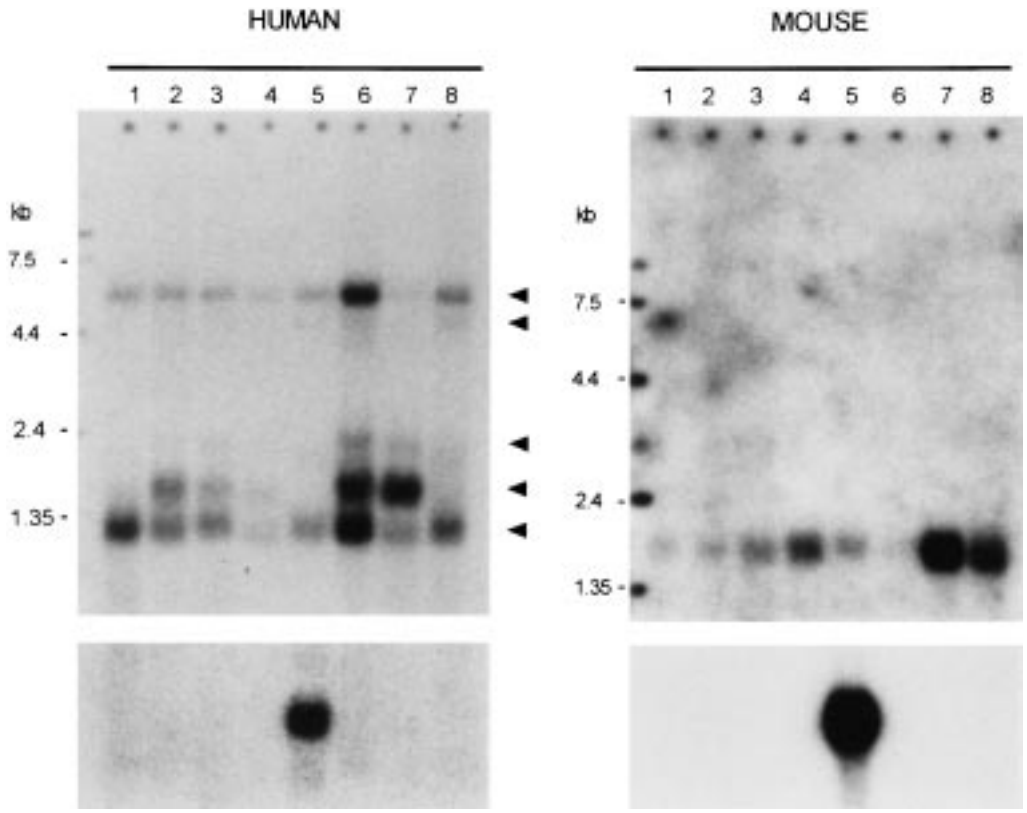


Fig. 2. Distribution of type I and II arginase mRNAs in human and mouse tissues. Northern blots (Clontech Laboratories, Inc.) containing approximately 2 μg of poly $A^+$ RNA from various human or mouse tissues were probed sequentially with human type II arginase cDNA (upper panel) and rat type I arginase cDNA (Kawamoto et al., 1987) (lower panel). The blots were stripped between probings. The faint dots at the top of the upper panels indicate the origin of each lane and the mobilities of RNA size markers are indicated on the left. Key to lanes in left panel: (1) heart, (2) brain, (3) placenta, (4) lung, (5) liver, (6) skeletal muscle, (7) kidney, (8) pancreas. Key to lanes in right panel: (1) heart, (2) brain, (3) spleen, (4) lung, (5) liver, (6) skeletal muscle, (7) kidney, (8) testis.

## 2. Results and discussion

### 2.1. Analysis of arginase sequences

The 1936-bp cDNA encoding human type II arginase contains 16 bp of 5′ untranslated sequence, a 1062-bp open reading frame, 788 bp of 3′ untranslated sequence and a 70-bp polyA tract. The open reading frame encodes a peptide of 354 amino acids which is 32 residues longer than human type I arginase, due to the presence of additional residues at both the N and C termini of type II arginase (Fig. 1). The additional N-terminal residues are probably involved in the mitochondrial localization of type II arginase, in contrast to type I arginase which is cytosolic. Like other mitochondrial matrix proteins (Hendrick et al., 1989; von Heijne et al., 1989), the N terminus of human type II arginase lacks acidic residues and has several arginine, leucine and serine residues. Human type II arginase is more closely related to *Xenopus* type II arginase (71% identity) than to human type I arginase (58% identity). This supports a previous suggestion (Patterson and Shi, 1994) that the two arginases are the consequence of a gene duplication event that occurred before mammals and amphibians diverged. Twenty-five residues are absolutely conserved among fifteen arginases from a wide variety of species (Fig. 1), including three histidines and four aspartates shown to be important for catalysis and stability of the binuclear metal center (Cavalli et al., 1994; Kanyo et al., 1996). The functions of the remaining conserved residues have yet to be determined. If the *Arabidopsis* arginase sequence is excluded from the comparison, however, an additional 13 residues are conserved among all the remaining arginases (Fig. 1). The total number of conserved residues identified in the present alignment is less than the 43 conserved residues

human (type I; Haraguchi et al., 1987), rat (type I; Kawamoto et al., 1987), *Rana catesbeiana* (type I; Iwase et al., 1995), *Saccharomyces cerevisiae* (Sumrada and Cooper, 1984), *Schizosaccharomyces pombe* (arginase 1; van Huffel et al., 1994), *Bacillus subtilis* (Gardan et al., 1995), *Bacillus caldovelox* (Bewley et al., 1996), *Agrobacterium tumefaciens* plasmid (Schrell et al., 1989), *Brucella abortus* (GenBank accession No. U57319), *Neurospora crassa* (SwissProt database, accession Argi_Neucr), *Coccidiodes immitis* (Pan et al., 1995), *Arabidopsis thaliana* (Krumpelman et al., 1995).

identified in a recent analysis of arginase sequences (Bewley et al., 1996), probably reflecting the fact that the present analysis included a larger number of arginase sequences representing a broader range of species than in the previous report.

### 2.2. Tissue-specific expression of type I and type II arginase genes

Type II arginase mRNA is present in virtually all human and mouse tissues tested (Fig. 2). The pattern of type II arginase mRNA expression contrasts sharply with that of type I arginase mRNA, which is represented by a single mRNA species and is expressed abundantly in liver, with little or no expression in other tissues. Surprisingly, at least five mRNA species hybridizing to type II arginase cDNA are found in the human RNA samples whereas only a single type II arginase mRNA species is found in the mouse. This raises the possibility that the multiple type II arginase mRNAs in humans arise from differential RNA processing or usage of alternative promoters. Although all human type II arginase mRNAs are of sufficient length to encode full-length type II arginase, the functional significance of the multiple mRNAs remains to be determined. Both type I and II arginase mRNAs were found in the liver RNA samples. So far as we are aware, this is the first clear evidence that both arginase isoforms can be expressed in liver. Although the identity of the hepatic cells expressing type II arginase is not known, it is likely that the type II arginase mRNA in this sample represents expression in nonparenchymal cells.

Although skeletal muscle has arginase activity (Pardridge et al., 1982), it is surprising that the abundance of type II arginase mRNAs in human skeletal muscle is as great as in human kidney, which is usually considered to be one of the sites of greatest type II arginase activity. This differs significantly from the mouse, where there is little or no type II arginase mRNA in skeletal muscle. Whether this represents a species difference or merely differences in the type of muscle sampled for the two species is not known. There is a high level of type II arginase expression in mouse testes, possibly to provide substrate for the synthesis of polyamines required for spermatogenesis.

## 3. Concluding remarks

Sequences of the type II arginases, combined with the recently determined tertiary structure of the rat type I arginase (Kanyo et al., 1996), will be useful in determining the basis for the differences in enzymatic properties between the type I and II arginases. The cDNA encoding the human type II arginase also will be essential for elucidating the physiologic functions of type II arginase during mammalian development, in different tissues and in disease states.

After this manuscript was submitted, another group reported the isolation of a cDNA clone with a coding sequence for human type II agrinase identical to that described here (Gotoh et al., 1996).

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