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Biochimica et Biophysica Acta 1509 (2000) 7–13

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

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Rapid report

Structure and function of ATA3, a new subtype of amino acid transport system A, primarily expressed in the liver and skeletal muscle

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Received 20 September 2000; accepted 4 October 2000

Abstract

To date, two different transporters that are capable of transporting α-(methylamino)isobutyric acid, the specific substrate for amino acid transport system A, have been cloned. These two transporters are known as ATA1 and ATA2. We have cloned a third transporter that is able to transport the system A-specific substrate. This new transporter, cloned from rat skeletal muscle and designated rATA3, consists of 547 amino acids and has a high degree of homology to rat ATA1 (47% identity) and rat ATA2 (57% identity). rATA3 mRNA is present only in the liver and skeletal muscle. When expressed in *Xenopus laevis* oocytes, rATA3 mediates the transport of α-[^{14}C](methylamino)isobutyric acid and [^{3}H]alanine. With the two-microelectrode voltage clamp technique, we have shown that exposure of rATA3-expressing oocytes to neutral, short-chain aliphatic amino acids induces inward currents. The amino acid-induced current is Na^+-dependent and pH-dependent. Analysis of the currents with alanine as the substrate has shown that the $K_{0.5}$ for alanine (i.e., concentration of the amino acid yielding half-maximal current) is 4.2 ± 0.1 mM and that the Na^+:alanine stoichiometry is 1:1.

Keywords: System A; Amino acid transporter A3; Electrophysiology; Liver; Skeletal muscle; Rat

Recently, two subtypes of amino acid transport system A, namely ATA1 (also known as GlnT) and ATA2 (also known as SAT2), have been cloned from rat and human tissues [1–6]. These transporters mediate the uptake of short-chain neutral amino acids in a Na^+-dependent manner. The ability to transport α-(methylamino)isobutyric acid (MeAIB), a specific substrate for amino acid transport system A, is a distinguishing feature of ATA1 and ATA2. Prior to the successful cloning of these transporters, functional studies provided little evidence for the existence of multiple subtypes of amino acid transport system A. System A was defined traditionally as a Na^+-dependent amino acid transport system specific for short-chain, neutral, aliphatic amino acids. In addition, the definition included the following: ability to transport MeAIB, pH sensitivity, ubiquitous expression in mammalian tissues, and sensitivity to hormonal regulation [7–9]. Surprisingly, when Varoqui et al. [1] cloned ATA1 as the first amino acid transporter capable of recognizing MeAIB as a substrate, this transporter was found to meet some, but not all, of

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0005-2736/00/$ – see front matter
PII: S0005-2736(00)00349-7

the criteria used to define system A. In rat, ATA1 is expressed primarily in the brain. In human, the expression of ATA1 is predominant in the placenta, heart and brain [2]. Since the classical, hormone-sensitive system A is known to be expressed ubiquitously, ATA1 does not represent this classical system A. Subsequently, ATA2 was cloned from rat and human tissues [3–6]. ATA2 is also able to mediate the transport of the system A-specific substrate MeAIB. But, unlike ATA1, ATA2 is expressed ubiquitously in rat and human tissues. It is therefore very likely that ATA2 represents the classical system A.

In this report, we describe the cloning of a third transporter capable of mediating the uptake of MeAIB. This transporter, designated ATA3, was cloned from rat skeletal muscle. ATA3 meets most of the functional criteria attributable to system A, but has a unique tissue distribution pattern. In rat, ATA3 is expressed abundantly in the liver and, to a much smaller extent, in the skeletal muscle.

The SuperScript plasmid system (Life Technologies, Rockville, MD, USA) was used to establish a unidirectional cDNA library with poly(A)$^+$mRNA isolated from rat skeletal muscle. Poly(A)$^+$mRNA was prepared by subjecting total RNA twice to oligo(dT)-cellulose affinity chromatography prior to use in a library construction. The cDNA products with sizes greater than 1 kb were separated by size fractionation and used for ligation at the *Sal*I-*Not*I site in the pSPORT1 vector.

A cDNA fragment of the recently cloned rat amino acid transport system N2 (unpublished), a Na^+- and H^+-coupled glutamine transporter similar to system N1 [10], was used as the probe in the screening of the rat skeletal muscle cDNA library. The screening was done under low stringency conditions as described previously [11–13]. Positive clones were identified and colonies purified by secondary screening. The cDNAs were sequenced using an automated Perkin-Elmer Applied Biosystems 377 Prism DNA sequencer. The sequence was analyzed using the GCG sequence analysis software package GCG version 10 (Genetics Computer Group, Madison, WI, USA).

A commercially available rat multiple tissue blot (Origene, Rockville, MD, USA) was used for Northern blot analysis to determine the tissue expression pattern of ATA3. The blot was hybridized sequentially under high stringency conditions with the cloned rat ATA3 cDNA and human glyceraldehyde-3-phosphate dehydrogenase cDNA as the probes.

Xenopus laevis oocytes were used for heterologous expression of the cloned rat ATA3 for functional analysis. cRNA from the cloned cDNA was synthesized using the mMESSAGE mMACHINE kit (Ambion, Austin, TX, USA). The cDNA was linearized using *Not*I, and the cDNA insert was transcribed in vitro using T7 RNA polymerase in the presence of an RNA cap analogue. The resultant cRNA was purified by multiple extraction with phenol/chloroform and precipitated with isopropanol. Mature oocytes were isolated by treatment with collagenase A (1.6 mg/ml) and manually defolliculated, and maintained at 18°C in modified Barth's medium supplemented with 10 mg/l gentamicin [14]. Oocytes ware injected with 50 ng of cRNA. Oocytes injected with water served as control. The oocytes were used for uptake or electrophysiological studies 5–6 days after cRNA injection. Electrophysiological studies were done by the conventional two-microelectrode voltage clamp method [15–17]. Oocytes were perfused with a buffer (100 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, pH adjusted by mixing 3 mM HEPES, 3 mM MES, and 3 mM Tris) followed by the same buffer containing different amino acid substrates. The membrane potential was held steady at -50 mV. The uptake of [^{3}H]alanine and [^{14}C]MeAIB in control oocytes and rATA3-expressing oocytes was measured as described previously [18].

The newly cloned rat ATA3 cDNA is 1946 bp long (GenBank accession No. AF295535) and encodes a protein containing 547 amino acids (Fig. 1). At the level of the amino acid sequence, this clone bears significant homology to rat ATA1 (47% identity; 63% similarity) and rat ATA2 (57% identity; 68% similarity), two previously known subtypes of amino acid transport system A. Rat ATA3 is longer than rat ATA1 and rat ATA2 because of the additional amino acid sequence located in the middle of the protein. Hydropathy analysis indicates that rat ATA3 has ten or 11 putative transmembrane domains.

We have also isolated a second ATA3 clone from the rat skeletal muscle cDNA library. This clone is 3934 bp long, much longer than the first clone. But

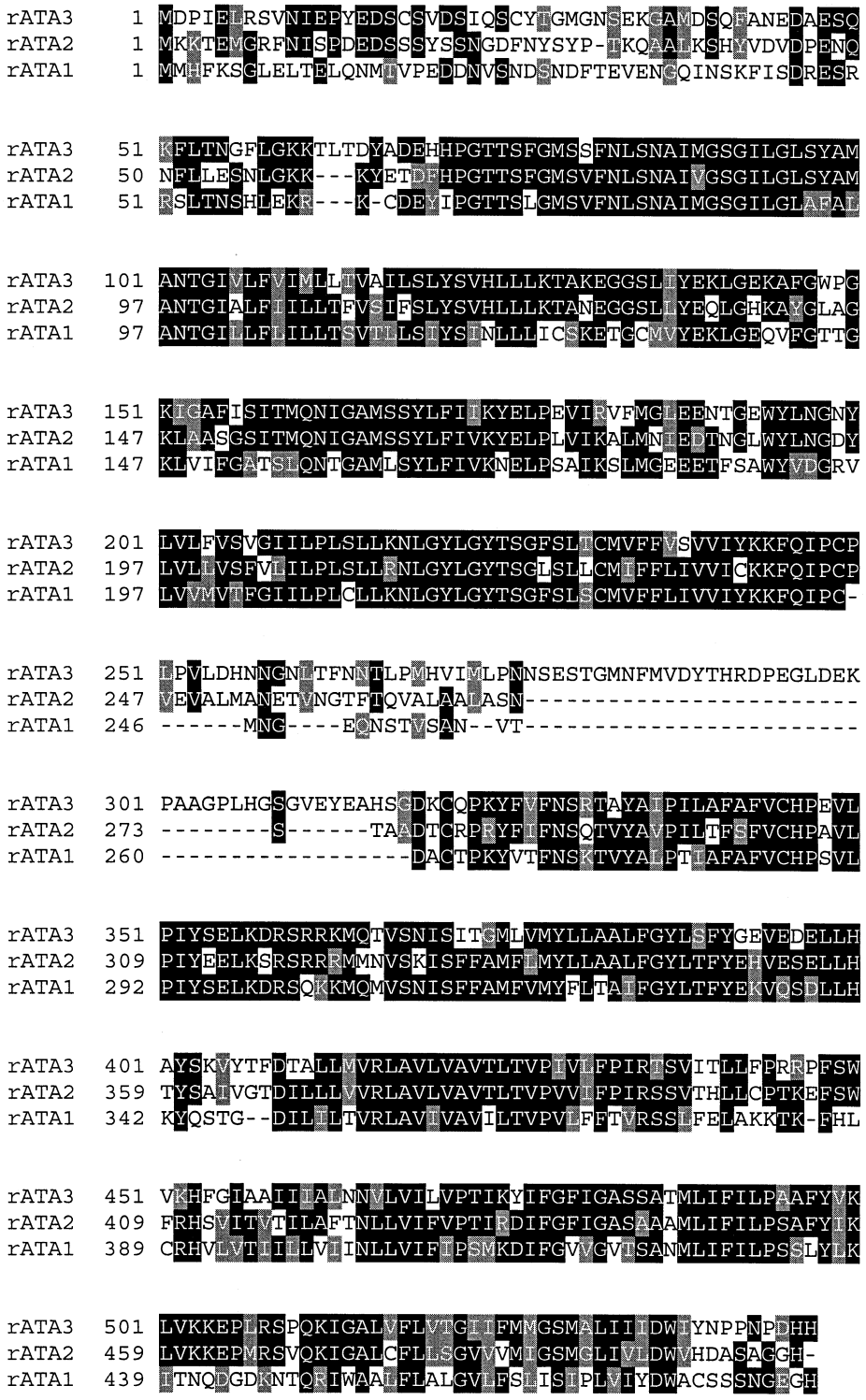

Fig. 1. Primary structure of rat ATA3. The amino acid sequence of rat ATA3 (rATA3) is compared with that of rat ATA1 (rATA1) and rat ATA2 (rATA2). The regions of sequence identity (dark shade) and similarity (light shade) among the three proteins are boxed.

sequencing of the second clone has revealed that it contains an open reading frame exactly identical to that of the first clone. The difference between the two clones lies in the 3′-untranslated region. These two clones apparently arise from alternative splicing.

The functional characteristics of rat ATA3 were studied by expressing the first clone in *X. laevis* oocytes heterologously. The transport function was evaluated by the uptake of radiolabeled amino acid substrates as well as by electrophysiological methods. Fig. 2 describes the uptake of [^{3}H]alanine and [^{14}C]MeAIB in water-injected oocytes and in oocytes expressing rat ATA3. The uptake of these two amino acids was severalfold higher in ATA3-expressing oocytes than in water-injected oocytes (alanine, 13-fold; MeAIB, 2.2-fold). Since MeAIB is considered a specific substrate for system A, the new transporter clone must represent a subtype of amino acid transport system A. Therefore, we have named this transporter ATA3 (amino acid transporter A3).

We then studied the transport function of rat ATA3 in oocytes by electrophysiological means. Perfusion of ATA3-expressing oocytes with 5 mM alanine induced marked inward currents in the presence of NaCl. The currents were negligible under identical conditions in water-injected oocytes, showing that alanine-induced currents were associated with rat ATA3. The ATA3-specific alanine-induced currents were Na^+-dependent because the magnitude of the currents was reduced markedly when the studies were conducted in the presence of *N*-methyl-D-glucamine chloride in place of NaCl. Cl^- had no role in the transport function as is evident from the lack of

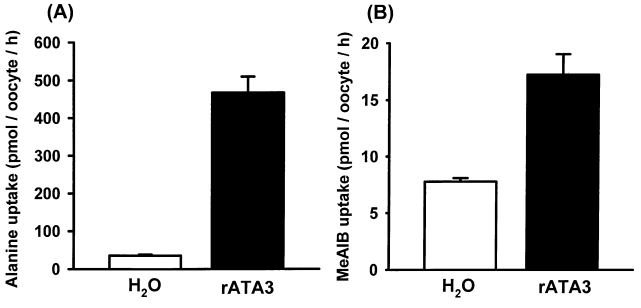

Fig. 2. Uptake of alanine (A) and MeAIB (B) by rATA3-expressing *X. laevis* oocytes. Uptake of [^{3}H]alanine (200 μM) and [^{14}C]MeAIB (50 μM) was measured in water-injected oocytes and in rATA3 cRNA-injected oocytes for 1 h. The uptake medium (pH 8.0) contained NaCl.

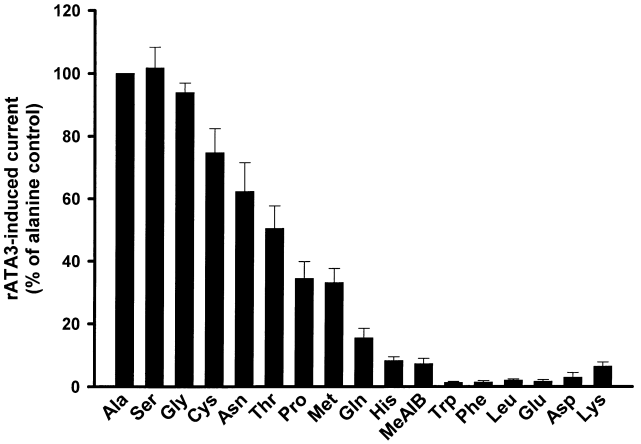

Fig. 3. Substrate specificity of rATA3. rATA3 was expressed in *X. laevis* oocytes and currents induced by various amino acids (5 mM) were measured at pH 8.0 using the two-microelectrode voltage clamp technique. Values are mean ± S.E. from three oocytes and given as percentage of alanine-induced current. The value for alanine-induced current in three oocytes was 93 ± 19 nA (100%).

any significant change in the alanine-induced currents when studied in the presence of sodium gluconate instead of NaCl. The ATA3-specific alanine-induced currents were, however, pH-dependent, the magnitude of the currents increasing markedly as the pH of the perfusion buffer increased from 5.5 to 8.5 (data not shown). These characteristics of rat ATA3 are similar to those of ATA1 and ATA2.

The substrate specificity of rat ATA3 was then studied by assessing the ability of various amino acids (5 mM) to induce currents in ATA3-expressing oocytes in the presence of NaCl. The relative magnitudes of the currents induced by several amino acids are given in Fig. 3 as percentage of alanine-induced currents (100%). The zwitterionic amino acids glycine, serine, cysteine, asparagine and threonine were excellent substrates for rat ATA3. The magnitude of the currents induced by these amino acids was $>50\%$ of alanine-induced currents. Proline, methionine, glutamine and histidine induced currents that were 15–30% of alanine control. MeAIB and lysine showed small but significant currents. The aromatic amino acids (phenylalanine, tryptophan, and tyrosine) and the anionic amino acids (aspartate and glutamate) were almost totally ineffective in inducing currents.

We then studied the characteristics of ATA3 transport function using alanine as the substrate. Alanine-

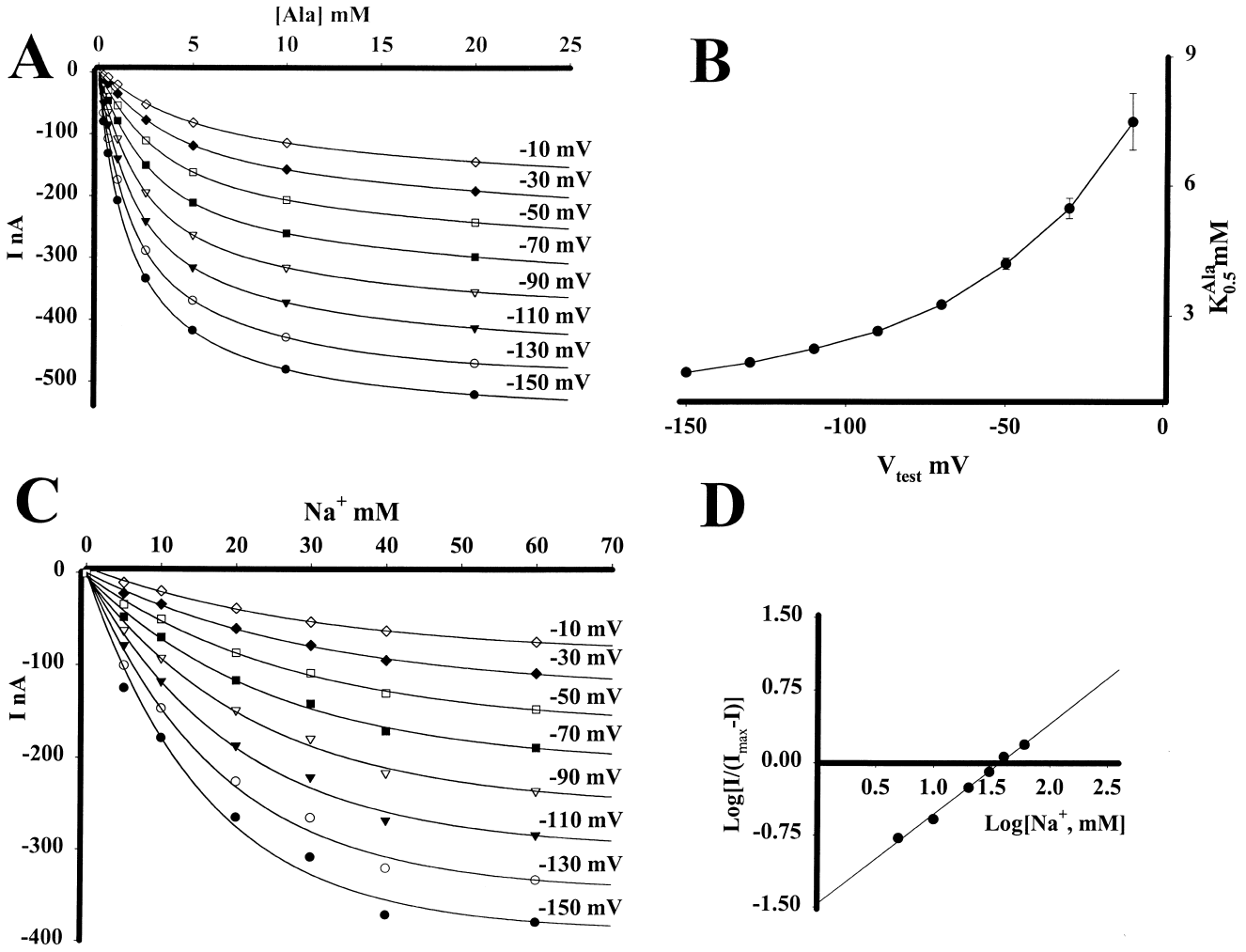

Fig. 4. Electrophysiological characteristics of rat ATA3. (A) Alanine-induced currents were measured in rat ATA3-expressing oocytes at pH 8.0 in the presence of NaCl. Concentration of alanine was varied in the range of 0.25–20 mM. (B) Influence of membrane potential on $K_{0.5}$ for alanine (i.e., alanine concentration needed for induction of half-maximal current). (C) Alanine-induced currents were measured in rat ATA3-expressing oocytes at pH 8.0. Alanine concentration was constant at 5 mM whereas Na^+ concentration was varied in the range of 5–60 mM. Osmolality and Cl^- concentration were maintained constant by appropriately altering the concentration of NaCl and *N*-methyl-D-glucamine chloride. (D) Hill plot for Na^+ activation of alanine-induced currents at −50 mV.

induced currents were saturable with alanine concentration at all membrane potentials studied (−10 to −150 mV) (Fig. 4A). These data were analyzed by Michaelis-Menten kinetics and $K_{0.5}$ values for alanine (i.e., concentration of alanine needed for the induction of half-maximal current) were calculated. The $K_{0.5}$ was about 4.2 ± 0.1 mM at −50 mV. This value was influenced markedly by the membrane potential (Fig. 4B). Hyperpolarization of the membrane decreased $K_{0.5}$ whereas depolarization increased $K_{0.5}$. Alanine-induced currents were also related hyperbolically to Na^+ concentration at all membrane potentials studied (Fig. 4C). These data were analyzed by Hill kinetics and the Hill coefficient for the activation of ATA3 transport function by Na^+ was in the range of 0.9–1.2 depending on the membrane potential. At −50 mV, the Hill plot was linear with a Hill coefficient of 0.96 ± 0.12 (Fig. 4D). These data show that the substrate affinity of ATA3 is influenced by membrane potential and that the Na^+:alanine stoichiometry is 1:1.

Northern blot analysis indicated that ATA3 mRNA is expressed most predominantly in the liver (Fig. 5). Skeletal muscle also expressed detectable

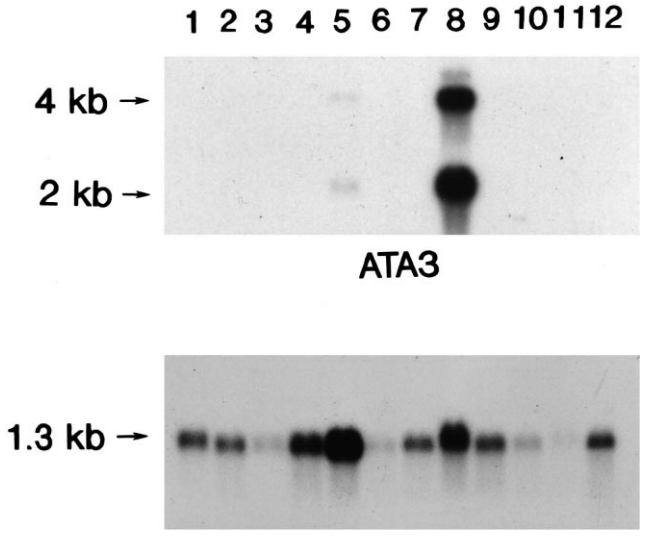

Fig. 5. Northern blot. A commercially available multiple tissue blot was used to analyze the tissue distribution pattern of ATA3 mRNA in the rat. The blot was hybridized sequentially with rat ATA3 cDNA (top panel) and human glyceraldehyde-3-phosphate dehydrogenase cDNA (bottom panel). The sizes of the hybridization signals are indicated. Lanes 1–12 represent brain, thymus, lung, heart, skeletal muscle, stomach, small intestine, liver, kidney, spleen, testis, and skin, respectively.

levels of the transcripts. The expression in all other tissues such as brain, thymus, lung, heart, stomach, small intestine, kidney, spleen, testis, and skin was below detectable levels. Two different transcripts were detected with sizes of 2.0 and 4.0 kb that correspond to the sizes of the two clones described in the present study.

In summary, we have cloned rat ATA3, a new subtype of amino acid transport system A, from rat skeletal muscle. This new transporter is expressed at detectable levels only in the liver and skeletal muscle. The levels of mRNA in the liver are, however, severalfold higher than in the skeletal muscle. Functionally, this transporter is similar to ATA1 and ATA2 except for the comparatively lower substrate affinity of ATA3. The tissue expression pattern of ATA3 is unique because ATA1 is expressed primarily in the brain, placenta and heart while ATA2 is expressed ubiquitously. This is the first report of the molecular characterization of an amino acid transport system A that is expressed abundantly only in the liver. It is therefore obvious that liver expresses at least two different subtypes of system A (ATA2 and ATA3). Functional studies have shown that the amino acid transport system A in the liver is subject to hormonal regulation. The transport system is known to be influenced by glucagon, insulin, starvation, and pregnancy [19–21]. Since ATA2 as well as ATA3 are likely to contribute to the amino acid system A activity in the liver, a physiologically important question arises as to the identity of the system A subtype that is regulated by hormones. Studies are underway in our laboratory to determine whether ATA2 and ATA3 are regulated differentially by hormones.

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