An ABC transporter homologous to TAP proteins[1]

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Abstract Polymerase chain reaction amplification of cDNA from rat intestine revealed the expression of a novel ABC transporter, TAPL (TAP-like). Subsequently, the protein sequence was deduced from the nucleotide sequence of cDNA carrying the entire coding region. TAPL is transcribed ubiquitously in various rat tissues. The protein, with 762 amino acid residues, has potential transmembrane domains, and an ATP-binding domain in its amino and carboxyl terminal regions, respectively, and is highly homologous to TAP1 and TAP2 (transporters associated with antigen presentation/processing): pairwise comparisons with TAPL demonstrated 39 and 41% of the residues are identical, respectively. These numerical values are essentially the same as that for TAP1 and TAP2 (39%), and the hydropathy profiles of TAPL, TAP1 and TAP2 are quite similar. The similarity among these three proteins suggests that they could be derived from a common ancestral gene. Furthermore, we found that there is a potential splicing isoform, sharing the amino terminal 720 amino acid residues of TAPL.

Key words: ABC transporter; TAP; Peptide transport; Antigen presentation

1. Introduction

Pump ATPases are intrinsic membrane proteins, which transport solutes across the membranes coupled with ATP hydrolysis [1]. They are widely distributed in prokaryotes and eukaryotes, and function in various cellular processes [2]. The ABC transporters, a family of pump ATPases, translocate a variety of molecules from peptides and proteins to organic compounds and inorganic ions [3]. Members of this family have one or two ATP-binding domain(s) comprising approximately 200 well conserved residues alternating with one or two transmembrane domains [4].

Recently, the entire yeast genomic structure was determined [5], and the 29 genes encoding proteins with the ATP-binding domain (designated to the ATP-binding cassette) characteristic of ABC transporters were found among approximately 6000 genes [5,6]. Considering the genome size in higher eukaryotes [7], it can be assumed that many more genes for ABC transporters could be found in mammals. Although the ATP-powered transport mechanism is not well known, most of the deficient functions of mammalian ABC transporters are related to diseases [6]. Furthermore, the clinical problem of drug resistance is ascribed to the function of ABC transporters such as P-glycoprotein and the transporters in human pathogens [6].

The aim of this study is to find novel mammalian ABC transporters in the intestine by means of homology cloning, since we are interested in the secretory functions of the gastrointestinal tract [8,9]. cDNA derived from rat intestinal mRNA was subjected to PCR with degenerate primers for conserved sequences, Walker A and Walker B motifs [10], of the ATP-binding domain of ABC transporters. Interestingly, we succeeded in finding a transcript closely related to TAP1 and TAP2, both of which participate in peptide transport from the cytoplasm to the lumen of the endoplasmic reticulum and the presentation of peptide antigens by MHC class I [11]. The primary structure of the novel transporter was deduced and its characteristics are reported.

2. Materials and methods

2.1. cDNA cloning

Total RNA was extracted from tissues of 5 weeks old Sprague Dawley rats by the guanidine thiocyanate-CsCl method and then subjected to cDNA synthesis [12]. Degenerate primers 1 and 2 corresponding to the conserved sequences of the ATP-binding cassette region of ABC transporters [10] were synthesized (Fig. 1A). They were used to amplify the cassette region of the novel transporter by means of PCR [13] with cDNA from intestine; the conditions for the 1st and 2nd PCR were 30 cycles of denaturation (94°C, 1 min), annealing (48°C, 2 min), and extension (72°C, 3 min), and 30 cycles of denaturation (94°C, 1 min), annealing (55°C, 2 min), and extension (72°C, 3 min), respectively (Fig. 1B).

5′-Upstream and 3′-downstream sequences were amplified by RACE using kidney cDNA, since the cDNA in question seems to be abundant in this organ compared with in intestine. Primers 7–9 and phosphorylated random primers, and primers 13–16 and phosphorylated primer 12 were prepared for 5′-RACE using a 5′-Full RACE Core Set (Takara, Kusatsu, Japan). Primers 3 and 4, and primers 5 and 6 were prepared for 5′-RACE and 3′-RACE, respectively, using a 5′-RACE System (GIBCO BRL, Tokyo, Japan). cDNA carrying the entire coding region was obtained by PCR with primer pairs 11 and 20 then 11 and 21 (Fig. 1B). The PCR conditions for these experiments were 35 cycles of denaturation (94°C, 1 min), annealing (55°C, 2 min), and extension (72°C, 3 min). In all the PCR, preheating (94°C, 3 min) and post-incubation (72°C, 7 min) were carried out before and after successive cycle reactions, respectively.

2.2. DNA sequencing

Amplified DNA was analyzed by agarose gel electrophoresis (1%, (w/v) Takara Type L03) using TAE buffer (1×) [12]. The DNA fragment was ligated to the pCRII vector (Invitrogen, CA, USA) or the pGEM-T Easy vector (Promega, WI, USA). Both strands of the cloned DNA were sequenced by the dideoxy chain-termination method [14] using a Silver Sequence DNA Sequencing System (Promega) or a Shimadzu DNA Sequencer Model DSQ-1000L.

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[1] The nucleotide sequence data reported in this paper will appear in the DDBJ/EMBL/GenBank nucleotide sequence databases with the accession number AB027520.

Abbreviations: ABC, ATP-binding cassette; bp, base pairs; EST, expressed sequence tag; MHC, major histocompatibility complex; RACE, rapid amplification of cDNA ends; PCR, polymerase chain reaction; SDS, sodium dodecyl sulfate; TAP, transporter associated with antigen presentation/processing

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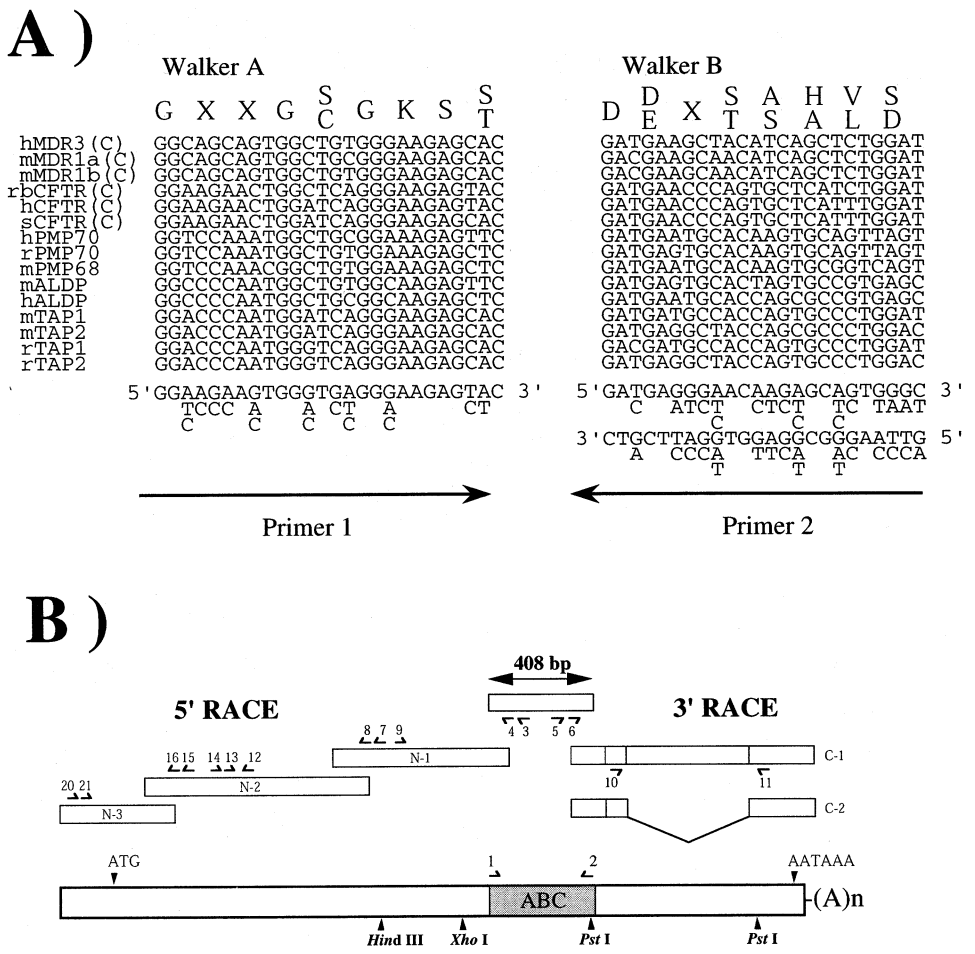

Fig. 1. Amplification of cDNA for a novel ABC transporter by PCR. A: Design of degenerate primers for PCR: The nucleotide sequences for the conserved amino acid sequence motifs (Walker A and Walker B [10]) in the ABC regions of ABC transporters are aligned. Degenerate primers 1 and 2 for PCR were designed from the aligned sequences; hMDR3 (M23234), mMDR1a (M30697), mMDR1b (M14757), rbCFTR (U40227), hCFTR (M28668), sCFTR (U20418), hPMP70 (M81182), rPMP70 (D90038/J05256), mPMP68 (X89569), mALDP (Z33637), hALDP (Z21876), mTAP1 (M55637), mTAP2 (M90459), rTAP1 (X57523) and rTAP2 (X63854). The accession numbers in the GenBank database are shown in parentheses. The carboxyl terminal ABC regions (C) of MDR and CFTR were chosen in this study. h, human; m, mouse; r, rat; rb, rabbit; s, sheep. B: Strategy for amplification of cDNA for TAPL: cDNA was subjected to PCR with primers 1 and 2 shown in A. The amplified 408 bp fragment was sequenced, and 5′-RACE and 3′-RACE were further carried out using the primers indicated by arrows with numerical values. The resulting clones (N-1, N-2 and N-3 on 5′-RACE, and C-1 and C-2 on 3′-RACE) were sequenced. Finally, primers 11, 20 and 21 were used for PCR amplification of the cDNA carrying the entire coding region, whose nucleotide sequence was determined. The full length cDNA is schematically shown in the lower part with a potential initiation codon (ATG) and poly-A additional signal (AATAAA) [21]. Typical restriction enzyme sites and a poly-A tail are also shown. The dotted box corresponds to the ABC region sandwiched between the Walker A and Walker B sequence motifs.

2.3. Determination of the tissue distribution of TAPL mRNA and amplification of genomic DNA

PCR with primers 3 and 9 was carried out (35 cycles of denaturation (94°C, 1 min), annealing (60°C, 2 min), and extension (72°C, 3 min)). The annealing temperature was reduced to 55°C for PCR conditions with primers 10 and 11. The rat β-actin mRNA level was determined by PCR with primers S and A [15] under the conditions of 35 cycles of denaturation (94°C, 1 min), annealing (65°C, 2 min), and extension (72°C, 3 min). Primer pairs 11 and 20, then 11 and 21 were used as above to amplify the entire coding region of TAPLa and most of the coding region of TAPLb. After agarose gel electrophoresis, the specific DNA bands were visualized with ethidium bromide and then the images were processed with a FluorImager Model 595 (Molecular Dynamics, CA, USA). Rat genomic DNA was prepared [12] and partially digested with *Sau*3AI restriction enzyme. The fragments were subjected to PCR with primers 10 and 11 as above.

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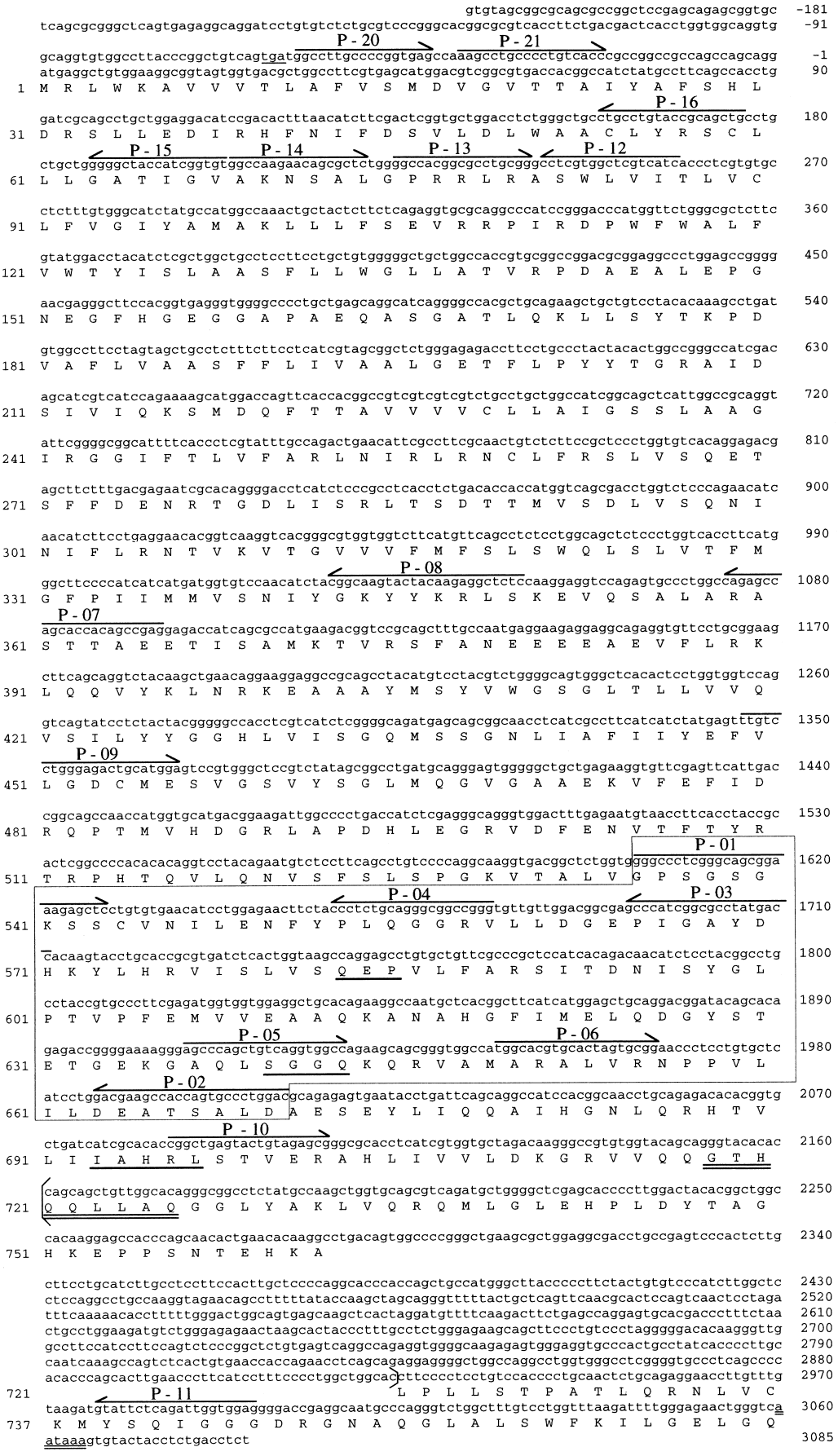
Fig. 2. Nucleotide sequence of cDNA for rat TAPL with the deduced amino acid sequence. Nucleotide sequences from all the clones shown in Fig. 1B were combined. Nucleotides are numbered on the right of each line from the first letter of the initiation codon, and numbers on the left are those of amino acid residues starting from Met-1. The initiator methionine codon immediately downstream of the *in frame* termination codon (underlined) was tentatively assigned. The poly-A additional signal is double-underlined. The primer sequences used for cloning (Fig. 1B) and determination of the tissue distribution of mRNA (Fig. 4) are indicated by arrows over the nucleotide sequence. The 763 bp sequence missing from clone C-2 (Fig. 1B) is in parentheses. The carboxy terminal residues deduced from clone C-2 are also shown downstream of the parentheses. Amino acid sequences characteristic of the cluster II ABC transporter subfamily [6] are underlined. The amino acid sequence identical with that of rat MDR2 [20] is double-underlined.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2.4. Chemicals

Restriction enzymes were obtained from New England Biolab (MA, USA), Takara, Toyobo (Osaka, Japan), or Nippon Gene (Toyama, Japan). T4 DNA ligase was from Takara or Toyobo, and Taq polymerase from Nippon Gene or Perkin Elmer (NJ, USA). The PCR primers were purchased from Sawaday (Tokyo, Japan) or GIBCO BRL. All other chemicals used were of the highest grade commercially available.

3. Results

3.1. cDNA cloning of a novel ABC transporter, TAPL

cDNAs derived from rat intestine total RNA were subjected to PCR with degenerate primers (Fig. 1A) for the conserved ATP-binding cassette region of ABC transporters. The amplified DNA fragments of the expected length (about 400 bp) were cloned and sequenced. A novel ATP-binding cassette sequence (408 bp), which is closely related to that of TAP2 [16], was identified together with those for MDR1b (one amino terminal cassette sequence and 16 carboxyl terminal cassette sequences) [17]. We named the novel clone TAPL (TAP-like). We carried out successive PCR using cDNA prepared from kidney mRNAs followed by amplification of cDNA covering the entire coding region of TAPL (Fig. 1B), since the mRNA level of TAPL seemed to be higher in kidney than in intestine. The nucleotide and deduced amino acid sequences of TAPL are shown in Fig. 2.

TAPL, with 762 amino acid residues, is a half size ABC transporter, whose ATP-binding cassette region is located on the hydrophilic carboxyl terminal side. The ^{583}Gln-Glu-Pro and ^{640}Ser-Gly-Gly-Gln motifs in the ABC region, and the ^{693}Ile-Ala-His-Arg-Leu motif downstream of Walker B are characteristic of the cluster II ABC transporter subfamily [6].

3.2. Similarity of TAPL to TAP

TAPL exhibits significant homology with TAP1 and TAP2 (Fig. 3). Pairwise comparison demonstrated that 39 and 40% of the residues are identical, respectively, and 61 and 63% of the residues are conserved, if we consider the conservative substitutions [18]. Comparison between TAP1 and TAP2 showed that 39 and 60% residues are identical and conserved, respectively. Such similarity suggests that the three proteins might have evolved from a common ancestral gene and might have closely related functions.

TAPL, TAP1 and TAP2 show similar hydropathy profiles, and seven hydrophobic regions are obvious (not shown), suggesting that their higher ordered structures would also be correlated if not identical. TAP1 has eight potential transmembrane segments, as shown by an expression study on *Escherichia coli* [19].

3.3. Presence of isoforms of TAPL

As shown in Fig. 1B, two different but related clones were obtained on 3′-RACE. Clone C-1 has an extra 763 bp sequence. The two clones have the same nucleotide sequence

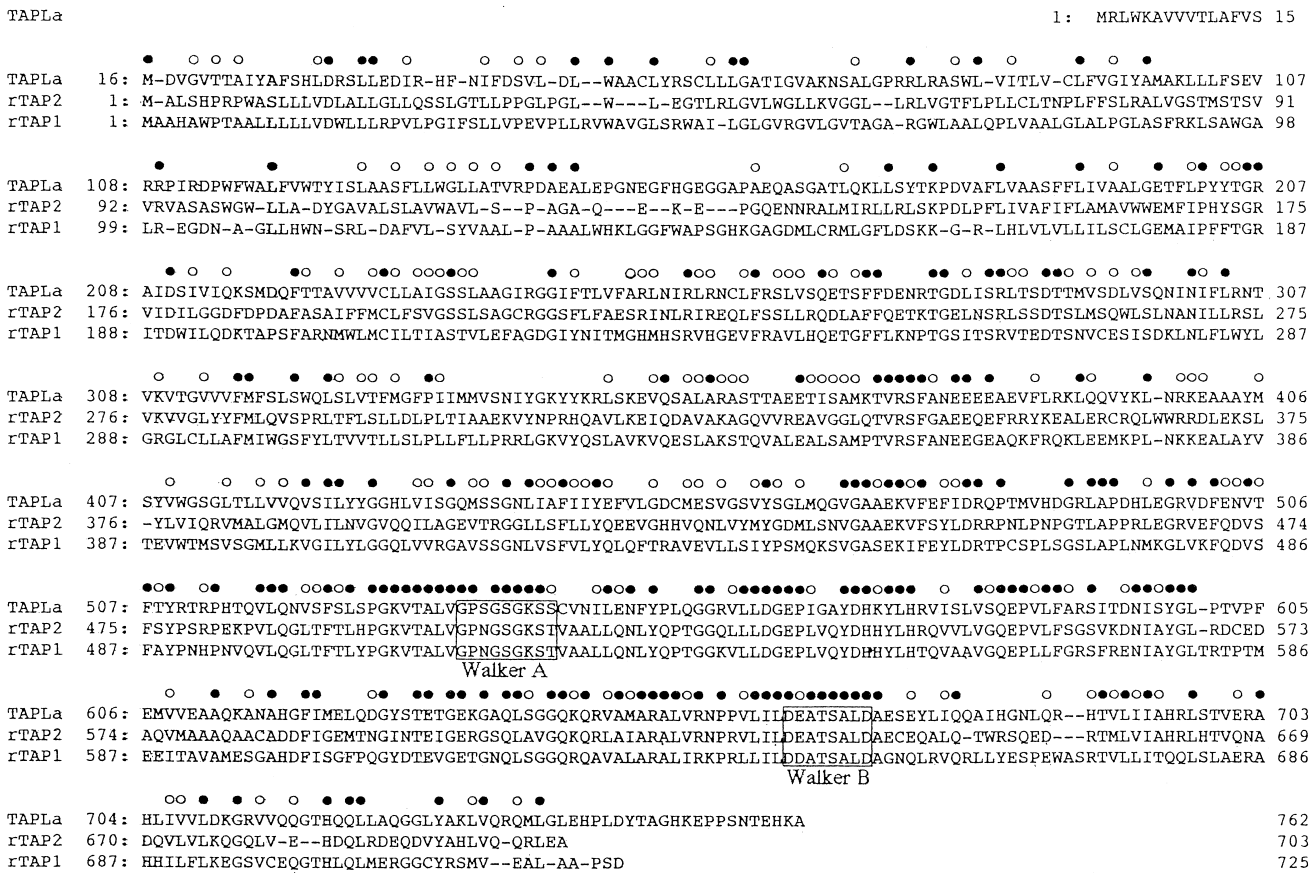

Fig. 3. Comparison of the amino acid sequence of TAPLa with those of TAP1 and TAP2. The amino acid sequence of TAPLa was compared with those of rat TAP1 and TAP2. Identical and conserved residues are denoted by closed and open circles, respectively. The ABC regions are located between the boxed Walker A and Walker B sequences.

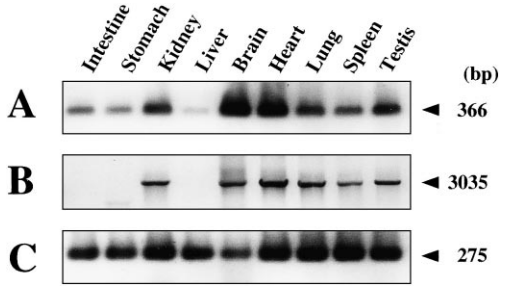

Fig. 4. Tissue distribution of TAPL mRNA. cDNAs were prepared from various rat tissues and then subjected to PCR. Primer pairs 3 and 9 (panel A), and 11 and 20, then 11 and 21 (panel B) were used for amplification. The products were analyzed by agarose gel electrophoresis. The mRNA level for β-actin was also determined as a control (panel C). The details are given in Section 2.

except that the 763 bp sequence is absent from clone C-2. They could be derived from potential isoform mRNAs producing proteins with different carboxyl terminal 42 (isoform 1 with 762 residues) and 46 (isoform 2 with 766 residues) amino acid residues, respectively (Fig. 2). We named these isoforms TAPLa and TAPLb, respectively. It should be mentioned that the carboxyl terminal nine residues of TAPLa (Gly^{718}-Gln^{726}), but not of TAPLb, are identical with those of rat MDR2 [20].

PCR amplification of rat genomic DNA with primers 10 and 11 (Fig. 1B) revealed only the 911 bp sequence identical to that of clone C-1 (data not shown), suggesting that clone C-2 could have been produced through splicing out of the 763 bp sequence from clone C-1 carrying the retained intron [21], although the potential splicing sequence (CA-AC termini) does not conform with the GT-AG rule [22]. However, a splicing sequence with unusual termini such as AT-AC was recently reported [23].

3.4. Tissue distribution of TAPL mRNAs

To determine the tissue distribution of TAPL, cDNAs prepared from various rat tissues were subjected to PCR. Primers 3 and 9 gave a 366 bp DNA fragment for all tissues examined (intestine, stomach, kidney, liver, brain, heart, lung, spleen and testis) (Fig. 4A), which covers Val^{450}-Asp^{570}. The expression of the two isoforms was also examined using primers 10 and 11. While the 911 bp DNA fragment corresponding to TAPLa (covering Leu^{697}-end) was reproducibly amplified, the 148 bp fragment corresponding to TAPLb was not (not shown). These results suggest that at least TAPLa is ubiquitously transcribed, although the amounts seem to be different in different tissues (Fig. 4A). Consistently, the entire sequence for TAPLa could be amplified from all tissues except intestine, stomach and liver (Fig. 4B). The sequence of TAPLb could not be obtained from any of the tissues, suggesting that the transcript of TAPLa could be abundant.

4. Discussion

Our results demonstrate that TAPL, which is closely related to TAP1 and TAP2, is ubiquitously transcribed in rat tissues. The sequence similarities of these three ABC transporters suggest that their genes have a common evolutional origin, and that they presumably have related functions. The TAP1 and TAP2 proteins hetero-dimerize on the endoplasmic reticulum membrane with their ABC regions facing cytoplasm, and then transport peptides onto the luminal side, where the peptides are bound to MHC class I molecules. Such complexes move to the plasma membrane and the bound peptides are presented to killer cell receptors as self and foreign antigens [11]. Defects in the functions of TAP1 and/or TAP2 cause human diseases such as Behçet's disease [24], multiple sclerosis [25], and bare lymphocyte syndrome type I [26]. Thus, it would be of interest to determine whether or not TAPL can transport peptides, substitute for TAP1 and TAP2, and be used therapeutically in patients with defects in TAP1 and TAP2.

It would also be interesting to determine homologous components for antigen presentation and peptide transporters from the view-point of chromosomal localization [27]. TAPL seems to be expressed widely in mammals, since a search of the EST database revealed that the registered sequences for mouse and man exhibited similarities to that of our TAPL. They had been analyzed in clones from kidney, and heart and brain, respectively. The fact that the human TAPL gene is not located on the same chromosome as that for TAP1 and TAP2 [27,28] could raise the possibility that TAPL is a peptide transporter not involved in antigen presentation. However, cells with an impaired TAP function still express low levels of cell surface MHC class I molecules, suggesting that alternative peptide transporter(s) might participate in the antigen presentation [29]. Such a TAP-independent route could be a focus as to the role of TAPL as well as other ABC transporters with unknown functions.

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