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A Half-Type ABC Transporter TAPL Is Highly Conserved between Rodent and Man, and the Human Gene Is Not Responsive to Interferon-γ in Contrast to TAP1 and TAP2[1]

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TAPL is a half-type ABC transporter with sequence similarity to TAP1 and TAP2 that is transcribed in various rat tissues [Yamaguchi, Y., Kasano, M., Terada, T., Sato, R., and Maeda, M. (1999) *FEBS Lett.* 457, 231–236]. Primary structures of the human and mouse orthologous counterparts were deduced from cDNAs cloned by means of polymerase chain reaction, and they were compared with that of the rat. The mammalian TAPLs (rat, mouse, and human) are highly conserved, since about 95% of the amino acid residues are identical between rodents and man. Phylogenetic analysis demonstrated that the evolutional rate of TAPL is much slower than those of TAP1 and TAP2, although TAPL could have diverged from an ancestor of TAP1 or that of TAP1 and TAP2. The TAPL-GFP fusion protein transiently expressed in Cos-1 cells was co-localized with PDI, suggesting that TAPL is inserted into endoplasmic reticulum membrane. The conservation of the peptide-binding motifs of TAP proteins in TAPL raises the possibility that the TAPL might be a peptide transporter. The gene for human TAPL is assigned to chromosome 12q24.31-q24.32, while those for TAP1 and TAP2 are located at the MHC locus of chromosome 6p21.3. Furthermore, the transcription of TAPL gene is not responsive to interferon-γ, in contrast to TAP1 and TAP2. These results indicate that the gene regulation of TAPL is different from those of TAP1 and TAP2.

Key words: ABC transporter, chromosome, GFP, interferon-γ, peptide transport, TAPL.

The ABC transporters with a conserved ATP-binding domain (named ABC, ATP binding cassette) are widely distributed in the membranes of various organisms from prokaryotes to eukaryotes (*1*). The canonical type of this family has two transmembrane and two ATP-binding domains arranged alternately. However, half-type transporters with a single transmembrane and single ATP-binding domain are also found, and they form a dimer to function. Complexes of four separate domains are often reported in bacterial ABC transporters (*2*). Although most ABC transporters are truly "membrane transport proteins," their functions are not restricted to the transport of proteins such as P-glycoprotein and histidine permease. Actually, CFTR is a Cl^- channel and SUR is a receptor-like protein (*2*). The ABC transporters are of interset from the viewpoints of medications and diseases, since they are responsible for drug resistance of cancer cells and infectious parasites as well as congenital human disorders (*3*).

Mammalian half-type transporters are known to be distributed in intracellular organelle membranes. Typically, TAP heterodimers, TAP1 and TAP2, are located on the ER membranes and transport peptide substrates into the ER lumen from the cytoplasm coupled to ATP hydrolysis. The function of TAP is important for cellular immunity, since peptides transported by TAP are used to present antigens on the cell surface MHC class I molecules (*4*). On peroxisomal membranes, there are as many as four half-type transporters (*5*) which seem to play roles in the biogenesis of peroxisomes and very long chain fatty acid metabolism (*6*).

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Abbreviations: ABC, ATP-binding cassette; BAC, bacterial artificial chromosome; bp, base pairs; BSA, bovine serum albumin; DMEM, Dulbecco's modified Eagle's medium; ER, endoplasmic reticulum; EST, expressed sequence tag; FISH, fluorescence *in situ* hybridization; GFP, green fluorescence protein; MHC, major histocompatibility complex; PDI, protein disulfide isomerase; RACE, rapid amplification of cDNA ends; PCR, polymerase chain reaction; SDS, sodium dodecyl sulfate; TAP, transporter associated with antigen processing; TAPL, TAP-like.

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The mitochondrial inner membrane also has a half-type ABC transporter involved in the iron homeostasis (*7*).

Recently we found an additional half type ABC transporter gene expressed in various organs of rat (*8*). This putative transporter has a high degree of amino acid sequence similarity to TAP1 and TAP2 and was thus named TAPL (TAP-like). Pairwise comparisons of TAPL, TAP1 and TAP2 demonstrated that about 40% of the amino acid residues are identical. In this study, we examined whether this novel transporter is regulated similarly to TAP proteins. Interestingly, we demonstrated that TAPL is a highly conserved protein, in contrast to TAP1 and TAP2. The chromosomal location, responsiveness to cytokine, and cytoplasmic localization of TAPL were also compared with those of TAP1 and TAP2, and the biological significance of TAPL is discussed.

MATERIALS AND METHODS

cDNA Cloning—HEK-293 cells (human embryonic kidney cell line) (*9*) and I-10 cells (mouse Leydig tumor cell line) (*10*) were cultured in two ϕ10 cm dishes, and total RNA was extracted by Isogen (Wako Chemicals) (*11*). The cDNAs for human and mouse TAPLs were amplified by means of RT-PCR. The first stranded cDNA was synthesized from 5 μg of RNA by SuperScript preamplification System (Gibco BRL) with oligo dT_{15} primer, and 1 μl of reaction product was subjected to PCR (*12*) with *Ampli Taq* (Nippon Gene). Various combinations of primers [designed from the sequences for rat (*8*) and registered ESTs] listed in Table I (A and B) were used. Sequence strategies are shown schematically in Fig. 1. In general, PCR conditions for the 1st and 2nd PCRs were 35 cycles of denaturation (94°C, 1 min), annealing (52–65°C, 1–2 min), and extension (72°C, 1–3 min). Either dimethyl sulfoxide (5%, v/v) or betaine (1.25 M) was added to the reaction mixture. In the present study, preheating (94°C, 3 min) and post-incubation (72°C, 7 min) were carried out before and after PCR, respectively.

DNA Sequencing—Amplified cDNAs were analyzed by agarose gel electrophoresis [1%, (w/v) Takara Type L03] using TAE buffer (1×) (*13*). The DNA fragment was ligated to the pCR™II vector (Invitrogen, CA) or pGEM-T Easy vector (Promega, WI). Both strands of cloned DNA were sequenced by the dideoxy chain-termination method (*14*) using a Silver Sequence DNA Sequencing System (Promega) or Shimadzu DNA Sequencer Model DSQ-1000L with Thermo Sequenase fluorescent labeled primer cycle sequencing kit (Amersham Pharmacia Biotech).

Construction of Expression Plasmid for TAPL-GFP Fusion Protein—cDNA of human TAPL was amplified by PCR using primers TM048 and TM049 (Table IC) carrying *Eco*RI and *Spe*I sites upstream of initiation codon and downstream of Glu-757 codon, respectively [30 cycles of denaturation (94°C, 1 min), annealing (48°C, 2 min), and extension (72°C, 3 min)]. The *Eco*RI–*Spe*I fragment (Met-1–Glu-757 codons) and the *Nhe*I–*Xho*I fragment (coding region of GFP) derived from pGFP-C1 (CLONTECH) were ligated into *Eco*RI and *Xho*I sites of pBluescript SKII(+). Then the *Eco*RI–*Xho*I fragment was inserted into the corresponding sites of mammalian expression vector pME18S. The resulting plasmid was named pMEhTAPL-GFP.

Indirect Fluorescence Analysis—Cos-1 cells (*15*) were grown for a day on glass coverslips in DMEM (GIBCO BRL) containing 7% (v/v) fetal bovine serum (JRH Bioscience). The expression plasmid for GFP (pGFP-C1) or TAPL-GFP (pMEhTAPL-GFP) was introduced by DEAE-dextran method (*16*) and cultured for 2 days. The empty plasmid vector (pME18S) was also introduced as a "Mock" experiment. Coverslips were processed and reacted (*17*) with the rabbit polyclonal-antibodies for PDI (1:200 dilution, Stress Gen), followed by incubation with Alexa Fluor 568–conjugated goat anti–rabbit IgG (1:200 dilution, Molecular Probes). Fluorescence of GFP and 2nd antibodies were monitored under a microscope (Olympus BX50).

Western Blotting—Soluble and membrane fractions were

TABLE I. **Primer sequences and their combinations for PCR.** Sequences of primers (A) and their combinations (B) for PCR amplification of human and mouse TAPL cDNAs were indicated. Each circled number in (B) corresponds to that on the amplified fragment schematically shown in Fig. 1. The primers for construction of expression plasmid pMEhTAPL-GFP and those for semiquantitative PCR to determine interferon-γ responsiveness of TAPL, TAP1, and TAP2 mRNAs are shown in (C). The base substitutions to introduce restriction enzyme sites are indicated by the underlines. The primers for β-actin were used to normalize the PCR amplification (h, human).

(A)

Primer	Sequence
YY004	5'- CCC GGC CGC CCT GCA GAG GG -3'
YY007	5'- CTC GGC TGT GGT GCT GGC TCT G -3'
YY008	5'- GAG AGC CTC TTG TAG TAC TTG CCG -3'
YY009	5'- TTG TCC TGG GAG ACT GCA TGG A -3'
YY019	5'- CTA CGG CAA GTA CTA CA -3'
YY020	5'- GGC CTT GCC CCG GTG AG -3'
YY021	5'- AAG CCT GCC CCT GTC ACC -3'
MK023	5'- ATG GCC TGC TGG ATC AG -3'
MK024	5'- CAG GTT GCC GTG GAT GG -3'
MK031	5'- TCA GCA CCC GCC CAC CTC C -3'
MK032	5'- GTG GGC ACA TCT GCC AGG -3'
MK033	5'- ACC GGG TCC TCT GCC ACC G -3'
MK034	5'- CAT GGC AGC TGG CGG GTG -3'
MK035	5'- GGA GCA AGT GGA AGG AGG C -3'
MK036	5'- CTG GAC CGC AGC CTC CTG GA -3'
MK044	5'- ATG AGA ACC GCA CAG GGG AC -3'
MK045	5'- CTG TAG GAC CTG TGT GTG GG -3'

(B)

No.	1st	2nd
①	1st:YY020-MK032	2nd:MK031-MK033
②	1st:YY020-YY007	2nd:YY020-YY008
③	1st:YY019-MK024	2nd:MK019-MK023
④	1st:YY019-YY004	2nd:YY019-YY004
⑤	1st:YY009-MK024	2nd:YY009-MK023
⑥	1st:YY020-MK034	2nd:YY021-MK035
⑦	1st:YY020-MK007	2nd:YY020-YY008
⑧	1st:MK036-YY008	
⑨	1st:MK044-MK045	
⑩	1st:YY019-YY004	2nd:YY019-YY004
⑪	1st:YY009-MK024	2nd:YY009-MK023

(C)

Gene	Primer	Sequence
hTAPL /GFP	TM048	5'- CCG AAT TCC AAC CAG CAG GAT G -3'
	TM049	5'- CTA CTA GTT CGT TGT GGC CAG CT-3'
hTAPL	MK031	5'- TCA GCA CCC GCC CAC CTC C -3'
	MK037	5'- CAG CCA CCA GAG CAG GAA GGA -3'
hTAP1	MK038	5'- GCT ACT TCT CGC CGA CTG GG -3'
	MK039	5'- CCC CAG TGC AGT AGC CTG GG -3'
hTAP2	MK040	5'- CCC GCC GCG GCT GAG CCA -3'
	MK042	5'- GGC ATG GGG GTC AAA ATC ACC -3'
h β-actin	h β actin-S	5'- GCA AGA GAT GGC CAC TGC CGC -3'
	h β actin-A	5'- GCT GAC AGG ATG CAG AAG GAG A -3'

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prepared (*18*) from Cos-1 cells transfected with expression plasmid for either GFP or TAPL-GFP as above, and aliquots were subjected to SDS polyacrylamide (10 or 17%, w/v) gel electrophoresis (*19*). The proteins were electro-blotted onto Immobilon P (MILLIPORE) (*20*). GFP or TAPL-GFP was reacted with the rabbit polyclonal-antibodies for GFP (1:2,000 dilution, Boehringer Mannheim), then detected by use of an Amersham ECL™ Western blotting analysis system with horseradish peroxidase-linked donkey anti–rabbit Ig (1:4,000 dilution). Protein was assayed with a Bio-Rad Protein Assay (*21*), with BSA (fraction V, Sigma) as a standard.

FISH—The BAC clone containing human TAPL gene was used as a probe for FISH. The FISH combined with replicated (pro)metaphase R-bands (direct R-banding FISH) was applied (*22*, *23*). For suppression of the repetitive sequences in the cosmid clone, we used about 2-fold excess human Cot-1 DNA (BRL) compared with the published method (*24*). Labeling, hybridization, rinsing and detection were performed in a routine manner. The image was captured using CytoVision (Applied Imaging). The BAC clone was isolated by hybridization (*13*) of the high-density filters of the library (Genome Systems) with probes covering from the 5′-noncoding region to Val-563 codon of human TAPL. The probes were radiolabeled with [α-^{32}P]-dCTP (110 TBq/mmol, Amersham Pharmacia Biotech)

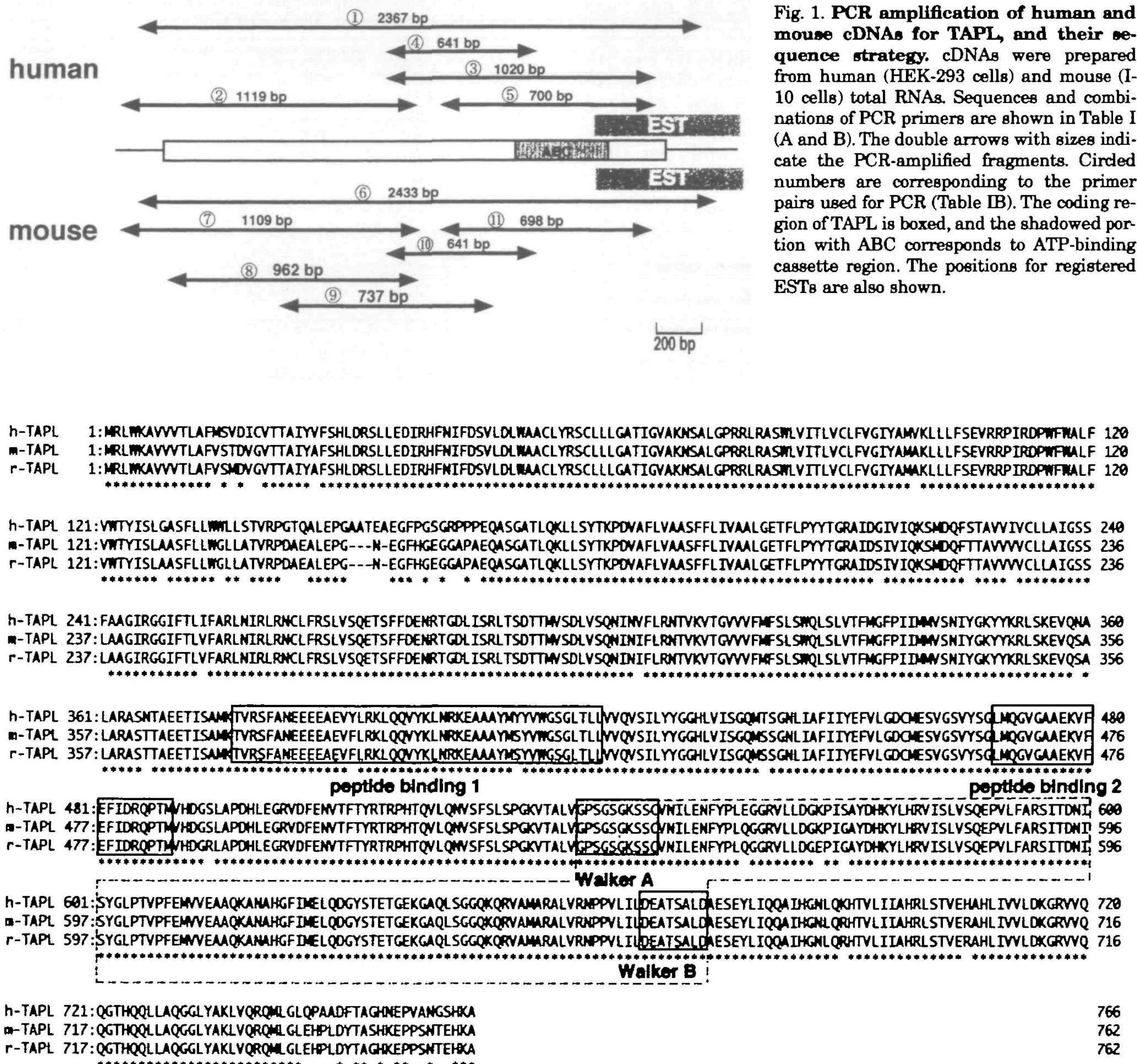

Fig. 1. **PCR amplification of human and mouse cDNAs for TAPL, and their sequence strategy.** cDNAs were prepared from human (HEK-293 cells) and mouse (I-10 cells) total RNAs. Sequences and combinations of PCR primers are shown in Table I (A and B). The double arrows with sizes indicate the PCR-amplified fragments. Circled numbers are corresponding to the primer pairs used for PCR (Table IB). The coding region of TAPL is boxed, and the shadowed portion with ABC corresponds to ATP-binding cassette region. The positions for registered ESTs are also shown.

Fig. 2. **Amino acid sequence comparisons of TAPL proteins.** The amino acid sequences of the human (h), mouse (m), and rat (r) TAPL proteins are aligned. Asterisks and hyphens show identical residues and gaps, respectively. The ATP binding regions between Walker A and Walker B motifs (*37*) are boxed with a dotted line. Two potential peptide-binding domains (peptide binding 1 and 2) compared with those for TAP1 and TAP2 (*25*) are also boxed. The residue numbers are shown on each line.

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using Random Primed Labeling Kit (TaKaRa). The templates were the rat cDNAs PCR-amplified with primer combinations 2 and 4 (Table I and Fig. 1B).

Induction Experiment with Interferon-γ—HeLa cells were cultured (1.8×10^6 cells/ϕ10 cm dish) in DMEM containing 7% fetal bovine serum for 1 day, then the medium was changed. Interferon-γ (R&D Systems) dissolved in 10 mM CH_3COOH and 0.1% (w/v) BSA was added (final 500,000 units/ml) into the fresh medium and further incubated for the indicated times in the text. HEK-293 cells were similarly cultured. Total RNA was isolated (2 dishes for each time point) (*11*) and cDNA was synthesized as described in the section "*cDNA Cloning.*" One microliter of reaction product was subjected to semiquantitative PCR analysis using primer pairs shown in Table IC. The denaturation and extension conditions [(94°C, 0.5 min) and (72°C, 0.5 min), respectively] are common for the amplification of human TAPL, TAP1, TAP2, and β-actin cDNAs, but cycle numbers and annealing conditions were changed [28 cycles (68°C, 0.5 min), 24 cycles (64°C, 0.5 min), 24 cycles (64°C, 0.5 min), and 16 cycles (60°C, 0.5 min), respectively]. After agarose gel electrophoresis (1.8%, w/v), the DNA bands were visualized with ethidium bromide and their images were processed on FluorImager Model 595 (Molecular Dynamics).

Chemicals—Restriction enzymes were obtained from New England Biolab (MA), TaKaRa Shuzo (Kyoto), Toyobo (Osaka), or Nippon Gene (Toyama). T4 DNA ligase was from TaKaRa or Toyobo. The PCR primers were purchased from GIBCO BRL. All other chemicals used were of the highest grade commercially available.

TABLE II. **Conservation of amino acid residues of TAPL, TAP1, and TAP2, and that of nucleotide residues in their coding sequences.** Identical amino acid residues (A.A.) between each pair of TAPL, TAP1, or TAP2 protein are indicated by percentage values. The conservation of nucleotide residues (N.A.) in their coding sequences is similarly shown in the parentheses. For the sequence data, see Fig. 2 and the legend to Fig. 3.

		Human/mouse	Human/rat	Mouse/rat
TAPL	A.A.	95	94	99
	(N.A.)	(89)	(89)	(95)
TAP1	A.A.	75	71	90
	(N.A.)	(75)	(74)	(90)
TAP2	A.A.	77	75	91
	(N.A.)	(80)	(78)	(92)

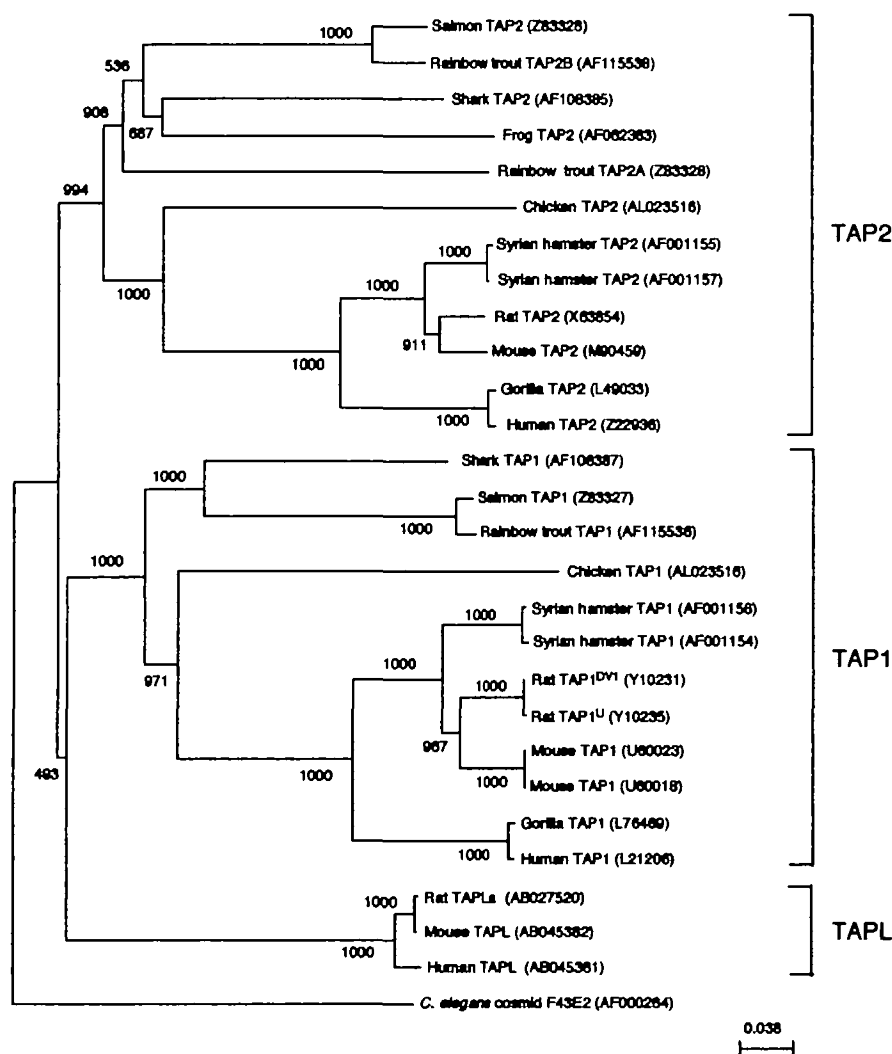

Fig. 3. **Phylogenetic tree of TAPL, TAP1, and TAP2.** The amino acid sequences were aligned using the CLUSTAL W program. The distance matrix thus obtained was used to construct a neighbor-joining tree (*38*). To assess the reliability of branching patterns, 1,000 bootstrap replications were performed. Numbers at the nodes indicate the bootstrap confidence level in permillage. The sources of the nucleotide sequences are from GenBank database and accession numbers are cited. Horizontal bar indicates genetic distance. A *C. elegans* ABC transporter whose gene is located in F43E2 region is also included, since it showed the highest homology to TAP.

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RESULTS

Mammalian TAPL Proteins Are Highly Conserved— Primary structures of human and mouse orthologous TAPLs were deduced by sequencing PCR amplified cDNAs from HEK293 cells and mouse I-10 cells, respectively, and compared with the rat TAPL. PCR primers are listed in Table I, and amplification of cDNA is shown schematically in Fig. 1. Mammalian TAPLs are half-type ABC transporters with about 750 amino acid residues and they are mutually well conserved (Fig. 2). Pairwise comparison demonstrated that 99% of the amino acid residues are identical between rat and mouse, and surprisingly as much as 95% of the residues are identical between rodents and man (Table II). This finding contrasts with that for the paralogous TAP proteins, since only 75% of the amino acid residues for TAP1 or TAP2 are identical between rodents and man in spite of the 90% identity between mouse and rat.

The phylogenetic analysis suggested that the origin of TAPL is old enough to trace back to the point of divergence of ancestral genes for TAP1 and TAP2 (Fig. 3). The ancestral gene for TAPL seems to have diverged immediately after the appearance of that for TAP1. Furthermore the evolutional rate of the TAPL gene is much slower than those for TAP1 and TAP2, since genetic distances between mammalian TAPLs are shorter than those for TAP1 and TAP2.

Intracellular Location of Transiently Expressed TAPL-GFP Fusion Protein—For TAP1 and TAP2 proteins, potential peptide-binding sites have been proposed (*25*). Such substrate binding domains are also conserved in mammalian TAPLs (Fig. 2). Fifteen residues from the start of the potential peptide-binding domain 1 of human TAPL are aligned together with the corresponding residues of human TAP1 and TAP2 (Table III). Actually, the sequences are well conserved among TAPL, TAP1, and TAP2. However, similar comparisons demonstrated that the sequences (*25*) are not conserved in the half-type ABC transporters of peroxisomal membrane (Table III).

Sequence similarity of TAPL to TAP1 and TAP2 prompted us to examine cytoplasmic location of TAPL. Since TAP1 and TAP2 form a heterodimer on the ER membrane (*26*), we examined whether the closely related TAPL is also located on the ER. For this purpose, we constructed an expression plasmid for TAPL-GFP fusion protein and introduced the plasmid into Cos-1 cells. The fluorescence emission from fusion protein was monitored under a microscope (Fig. 4A). The fluorescence was localized on the intracytoplasmic membrane. Probing the same culture with antibodies for PDI (Fig. 4B), a marker protein for ER (*27*), clearly indicated that the fluorescence from GFP was co-localized with that of the antibodies (Fig. 4C). When the free GFP was expressed, the fluorescence was distributed in the entire space of the cytoplasm (not shown).

We also carried out Western blotting analysis using antibodies for GFP. As shown in Fig. 5A (upper), a fusion protein of slightly smaller apparent molecular size (100 kDa) than the sum of the predicted values for human TAPL (84 kDa) and GFP (28 kDa) was detected in the membrane fraction, but not in the soluble cytoplasmic fraction. The increased amounts of membranes gave much stronger signal for the fusion protein (Fig. 5B). The mock and GFP-expressed cells had no protein of comparable size in either membrane or soluble fraction [Fig. 5, A (upper) and B]. The GFP was recovered in the soluble fraction (Fig. 5A, lower). These results suggest that at least the TAPL carrying GFP on its carboxyl terminus is inserted into ER membrane.

Chromosomal Location of Human TAPL—The chromosomal location of TAPL could not be determined precisely

TABLE III. **Comparison of potential peptide binding domain of TAPL with those of TAP1 and TAP2.** A portion of potential peptide-binding domain 1 of human TAPL is compared with those of human TAP1 and TAP2 (see legend to Fig. 3). Identical residues with human TAPL are open-boxed. The amino acid sequences located in the corresponding regions of peroxisomal membrane ABC transporters (PMP70 and ALDP; accession nos. X58528 and Z21876, respectively) are also shown. The residue numbers are indicated on the right.

hTAPL	:	TVRSFANEEEEAEVY	364-378 a.a.
hTAP1	:	TVRSFANEEGEAQKF	376-390
hTAP2	:	TVRSFGAEEHEVCRY	341-355
hPMP70	:	NSEEIAFYNGNKREK	275-288
hALDP	:	NSEEIAFYGGHEVEL	289-302

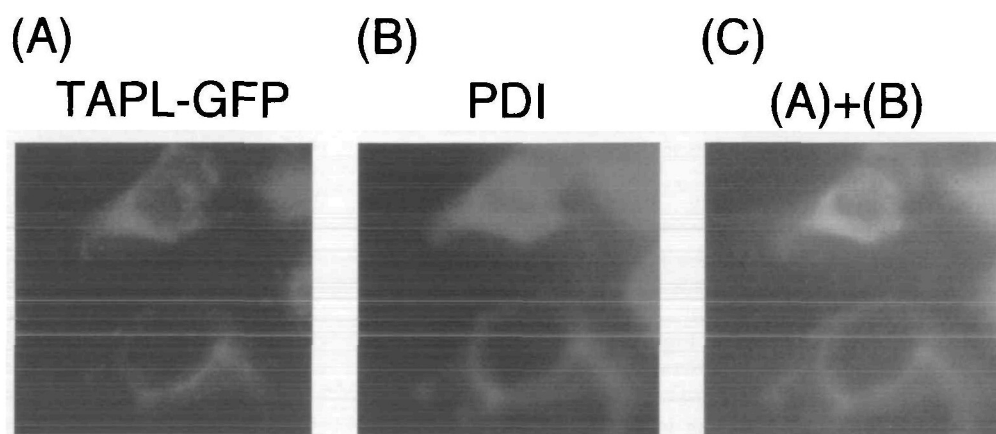

Fig. 4. **Detection of TAPL-GFP fusion protein transiently expressed in Cos-1 cells.** Cos-1 cells were plated on coverslips and grown for 1 day, and then expression plasmid for TAPL-GFP fusion protein or GFP was introduced. After 2 days' incubation, the cells were fixed, then reacted with polyclonal-antibodies for PDI followed by Alexa Fluor 568–conjugated goat anti-rabbit IgG. Green fluorescence of TAPL-GFP was monitored (A). The distribution of PDI (red fluoresence) in the same field as (A) was visualized (B). The combined image was obtained by monitoring the both fluorescences at the same time (C).

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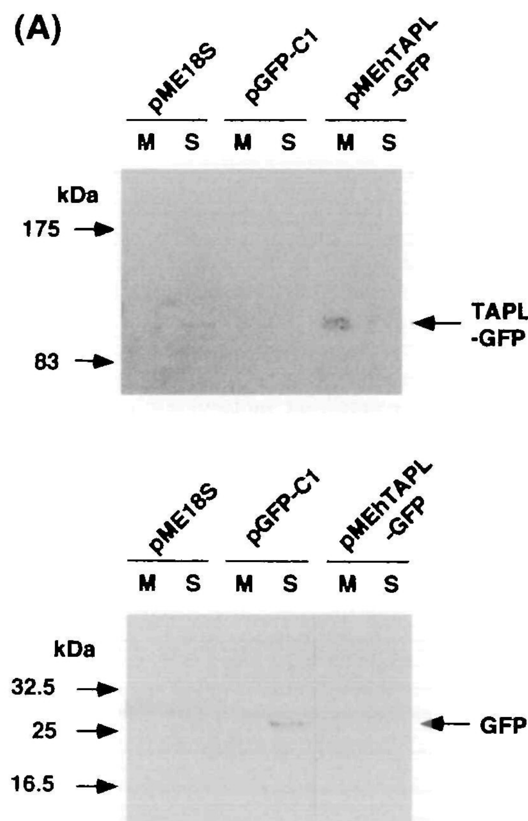

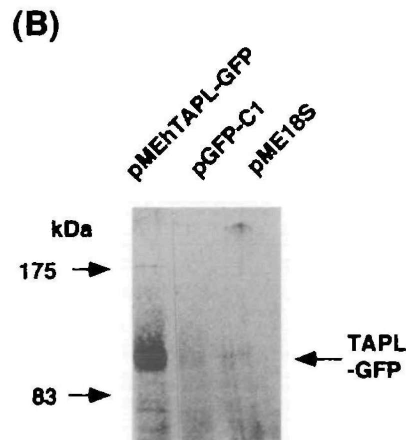

Fig. 5. **Presence of TAPL-GFP fusion protein on the membranes.** Expression plasmid for TAPL-GFP (pMEhTAPL-GFP) or GFP (pGFP-C1) was introduced into Cos-1 cells (5 × 10^5 cells/ϕ10 cm dish). After 2 days' incubation, cells from 2 dishes were scraped and homogenized. Soluble (S) and membrane (M) fractions were prepared as described in "MATERIALS AND METHODS." Protein samples (5 μg) were subjected to SDS–polyacryl amide gel electrophoresis and Western blotting. GFP moiety of TAPL-GFP (A, upper) and GFP (A, lower) were reacted with rabbit anti-GFP polyclonal antibodies, and then detected by means of ECL. The empty plasmid vector (pME18S) was also introduced as a "Mock" experiment. The increased amounts of membrane protein (20 μg) were analyzed in (B). The polyacrylamide gel concentration was 10% (w/v) for A (upper) and B, and 17% (w/v) for A (lower).

Fig. 6. **Chromosomal location of human TAPL.** Location of human TAPL gene is shown on R-banded metaphase chromosomes by FISH as described in "MATERIALS AND METHODS." The arrow indicates fluorescent signals on 12q24.31-q24.32. The BAC clone containing human TAPL gene was used as a probe.

from the human EST sequence (*28*). Thus we isolated a BAC clone carrying the human TAPL gene by hybridization with a human cDNA segment. The clone was subjected to FISH analysis and 50 typical R-banded metaphase plates were examined. As shown in Fig. 6, twin-spot signals due to the clone were localized on chromosome 12. No signal was detected on the other chromosomes. The result indicated that the gene for TAPL was located on the chromosome 12q24.31-q24.32. The genes related to diseases such as scapuloperoneal spinal muscular atrophy (12q24.1-q24.31), spinal muscular atrophy-4 (12q24.1-q24.33), brachydactyly typeC (12q24.1-q24.33), and Noonan syndrome 1 (12q22-q24ter) [MIM numbers 181405, 158590, 113100, and 163950, respectively, registered with Online Mendelian Inheritance in Man (OMIM)] are located near to or overlapping the TAPL locus of chromosome 12.

Effect of Interferon-γ on the mRNA Level of TAPL—The closely related TAP1 and TAP2 genes are responsive to interferon-γ (*29*). To determine whether the transcription of TAPL gene could be similarly regulated, we cultured human cells and studied the effect of interferon-γ on the mRNA level of TAPL. Semiquantitative PCR analysis demonstrated that TAP1 and TAP2 mRNA levels in HeLa cells were elevated significantly at 2.5 h after interferon-γ addition (Fig. 7, left). Their maximal levels (10- and 15-fold, respectively) were observed at 10 h, then gradually decreased. However, the mRNA levels of TAPL and β-actin were unchanged. Essentially the same results were obtained in HEK-293 cells (Fig. 7, right). These results suggest that the TAPL gene is not responsive to interferon-γ. Furthermore, the gene regulation of TAPL could be different from those of TAP1 and TAP2.

DISCUSSION

Sequence comparison of mammalian TAPLs demonstrated that they are highly conserved proteins, while closely related TAP1 and TAP2 (TAPs) are less conserved. The phylogenetic analysis suggest that the evolutional rate of the TAPL gene is slower than those of TAP genes, although the ancestral gene for TAPL seems to have diverged very soon after emerging of the common ancestor for TAP1 and

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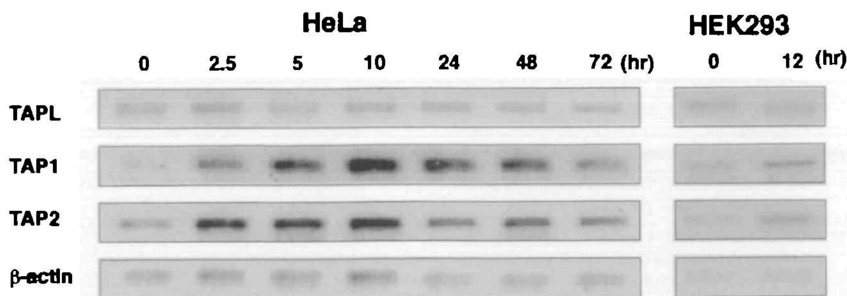

Fig. 7. **The mRNA levels of TAPL, TAP1, and TAP2 in human cultured cells upon adding interferon-γ in the medium.** HeLa cells were cultured in the presence of interferon-γ and total cellular RNA was prepared at the indicated times. The mRNA levels were determined by means of RT-PCR. The products (267, 321, and 582 bp for human TAPL, TAP1, and TAP2, respectively) were analyzed by agarose gel electrophoresis. The mRNA level for β-actin (278 bp) was also determined as a control. Primers used for PCR are listed in the Table IC. A similar experiment was carried out with HEK-293 cells and the amplification products at time zero were compared with those at 12 h of incubation.

TAP2 genes. These results suggest that the biochemical mechanism of TAPL could be closely related to the peptide transporter TAPs. The facts that the peptide-binding motifs proposed for TAP1 and TAP2 (*25*) are also conserved in TAPL and that the mammalian TAPLs are highly conserved suggest the potential role of TAPL as a peptide transporter recognizing a specific peptide substrate(s).

The gene regulation of TAPL seems to be different from those of TAP1 and TAP2, since the transcription of human TAPL gene was not affected by interferon-γ, in contrast to the great enhancement of the expression of TAP1 and TAP2 genes by this cytokine. Although an interferon-responsive element [(C/G)AAAAG(C/T)GAAACC] (*30*) and a STAT-binding element [TTCCC(A/G)(G/T)AA] (*31*) are located in the proximal promoter regions of TAP1 and TAP2 genes (*32*), such elements could not be found within 600 bp upstream from the initiation codon of TAPL gene (not shown).

The chromosomal location of human TAPL gene is also different from those of TAP genes; tandem TAP genes are located in the MHC class II region of chromosome 6 (*32, 33*), while that of TAPL is in chromosome 12. TAPL is not encoded in the chromosomal regions related to the adaptive immunity which are produced by gene duplications (*33*). Thus the physiological roles of TAPL are of interest from the viewpoints of secretion of biologically active peptide molecules or excretion of waste compounds produced during development and aging as well as from that of participation in the inflammatory activation through antigen presentation. It is also of interest to know whether the human diseases being mapped near the TAPL gene (12q24.31-q24.32) are associated with the alterations in the expression level and/or biochemical properties of this ABC transporter.

The loss of TAP1 molecule affected the assembly of TAP proteins on microsomal membranes (*34*), suggesting that TAP1 and TAP2 heterodimer is the predominant species in the native membrane, and that the monomer or homodimer of TAP2 is unstable. However, the TAP1 molecule is stable in the absence of TAP2 (*35*). TAPL-GFP fusion protein transiently overexpressed in Cos-1 cells where TAPL is transcribed (not shown) seems to be stably assembled on the ER membrane. Thus it is worthwhile to investigate whether TAPL could associate with TAP1 or TAP2 as well as itself, since TAPs are widely expressed in the tissues (*4*). The TAPL-GFP fusion protein co-localized with PDI, a marker protein for ER, is also detectable on the small peripheral vesicles, although in less significant amounts. These results suggest that TAPL might be a component of organelle membranes which traffic through the vesicular transport system (*36*).

REFERENCES

1. Hyde, S.C., Emsley, P., Hartshorn, M.J., Mimmack, M.M., Gileadi, U., Pearce, S.R., Gallagher, M.P., Gill., D.R., Hubbard, R.E., and Higgins, C.F. (1990) Structural model of ATP-binding proteins associated with cystic fibrosis, multidrug resistance and bacterial transport. *Nature* **346**, 362–365
2. Croop, J.M. (1998) Evolutionary relationships among ABC transporters in *Methods in Enzymology* (Ambudkar, S.V. and Gottesman, M.M., eds.) Vol. 292, pp. 101–116, Academic Press, New York
3. Decottignies, A. and Goffeau, A. (1997) Complete inventory of the yeast ABC proteins. *Nat. Genet.* **15**, 137–145
4. Heemels, M.-T. and Ploegh, H. (1995) Generation, Translocation, and presentation of MHC class I-restricted peptides. *Annu. Rev. Biochem.* **64**, 463–491
5. Liu, X.L., Janvier, K., Berteaux-Lecellier, V., Cartier, N., Benarous, R., and Aubourg, P. (1999) Homo- and heterodimerization of peroxisomal ATP-binding cassette half-transporters. *J. Biol. Chem.* **274**, 32738–32743
6. Valle, D. and Gärtner, J. (1993) Penetrating the peroxisome. *Nature* **361**, 682–683
7. Allikmets, R., Raskind, W.H., Hutchinson, A., Schueck, N.D., Dean, M., and Koeller, D.M. (1999) Mutation of a putative mitochondrial iron transporter gene (ABC7) in X-linked sideroblastic anemia and ataxia (XLSA/A). *Hum. Mol. Genet.* **8**, 743–749
8. Yamaguchi, Y., Kasano, M., Terada, T., Sato, R., and Maeda, M. (1999) An ABC transporter homologous to TAP proteins. *FEBS Lett.* **457**, 231–236
9. Graham, F.L., Smiley, J., Russell, W.C., and Nairn, R. (1977) Characteristics of a human cell line transformed by DNA from human adenovirus type 5. *J. Gen. Virol.* **36**, 59–74
10. Shin, S.-I. (1967) Studies on interstitial cells in tissue culture: steroid biosynthesis in monolayers of mouse testicular interstitial cells. *Endocrinology* **81**, 440–448
11. Chomczynski, P., and Sacchi, N. (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* **162**, 156–159
12. Saiki, R.K., Gelfand, D.H., Stoffel, S., Scharf, S.J., Higuchi, R., Horn, G.T., Mullis, K.B., and Erlich, H.A. (1988) Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* **239**, 487–491
13. Sambrook, J., Fritsch, E.F., and Maniatis, T. (1989) *Molecular Cloning. A Laboratory Manual*, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
14. Sanger, F., Coulson, A.R., Barrell, B.G., Smith, A.J.H., and Roe, B.A. (1980) Cloning in single-stranded bacteriophage as an aid to rapid DNA sequencing. *J. Mol. Biol.* **143**, 161–178
15. Jasin, M., de-Villiers, J., Weber, F., and Schaffner, W. (1985) High frequency of homologous recombination in mammalian

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cells between endogenous and introduced SV40 genomes. *Cell* **43**, 695–703
16. Yoshida, T., Sato, R., Mahmood, S., Kawasaki, S., Futai, M., and Maeda, M. (1997) GATA-6 DNA binding protein expressed in human gastric adenocarcinoma MKN45 cells. *FEBS Lett.* **414**, 333–337
17. Nakagawa, R., Sato, R., Futai, M., Yokosawa, H., and Maeda, M. (1997) Gastric GATA-6 DNA-binding protein: proteolysis induced by cAMP. *FEBS Lett.* **408**, 301-305
18. Maeda, M., Nishijima, M., Akamatsu, Y., and Sakakibara, Y. (1985) Alteration in the characters of CDP-choline synthetase and phospholipid-choline exchange enzyme upon choline starvation in Chinese hamster ovary cells. *J. Biol. Chem.* **260**, 5925–5930
19. Laemmli, U.K. (1970) Cleavage of structural proteins during the assembly of the bacteriophage T4. *Nature* **227**, 680–685
20. Towbin, H., Staehelin, T., and Gordon, J. (1979) Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. Natl. Acad. Sci. USA* **76**, 4350–4354
21. Bradford, M.M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**, 248–254
22. Takahashi, E., Hori, T., O'Connell, P., Leppert, M., and White, R. (1990) R-banding and nonisotopic *in situ* hybridization: precise localization of the human type II collagen gene (COL2A1). *Hum. Genet.* **86**, 14–16
23. Takahashi, E., Yamauchi, M., Tsuji, H., Hitomi, A., Meuth, M., and Hori, T. (1991) Chromosome mapping of the human cytidine-5′-triphosphate (CTPS) gene to band 1p34.1-p34.3 by fluorescence *in situ* hybridization. *Hum. Genet.* **88**, 119–121
24. Lichter, P., Ledbetter, S.A., Ledbetter, D.H., and Ward, D.C. (1990) Fluorescence *in situ* hybridization with Alu and L1 polymerase reaction probes for rapid characterization of human chromosome in hybrid cell lines. *Proc. Natl. Acad. Sci. USA* **87**, 6634–6638
25. Nijenhuis, M. and Hämmerling, G.J. (1996) Multiple regions of the transporter associated with antigen processing (TAP) contribute to its peptide binding site. *J Immunol.* **157**, 5467–5477
26. Raghavan, M. (1999) Immunodeficiency due to defective antigen processing: the molecular basis for type 1 bare lymphocyte syndrome. *J. Clin. Invest.* **103**, 595–596
27. Noiva, R. and Lennarz, W.J. (1992) Protein disulfide isomerase; a multifunctional protein resident in the lumen of the endoplasmic reticulum. *J. Biol. Chem.* **267**, 3553–3556
28. Allikmets, R., Gerrard, B., Hutchinson, A., and Dean, M. (1996) Characterization of the human ABC superfamily: isolation and mapping of 21 new genes using the expressed sequence tags database. *Hum. Mol. Genet.* **5**, 1649–1655
29. Ma, W., Lehner, P.J., Cresswell, P., Pober, J.S., and Johnson, D.R. (1997) Interferon-γ rapidly increases peptide transporter (TAP) subunit expression and peptide transport capacity in endothelial cells. *J. Biol. Chem.* **272**, 16585–16590
30. Tanaka, N., Kawakami, T., and Taniguchi, T. (1993) Recognition DNA sequences of interferon regulatory factor 1 (IRF-1) and IRF-2, regulators of cell growth and the interferon system. *Mol. Cell. Biol.* **13**, 4531–4538
31. Horvath, C.M., Wen, Z., and Darnell Jr., J.E. (1995) A STAT protein domain that determines DNA sequence recognition suggests a novel DNA-binding domain. *Genes Dev.* **9**, 984–994
32. Beck, S., Kelly, A., Radley, E., Ehurshid, F., Alderton, R.P., and Trowsdale, J. (1992) DNA sequence analysis of 66 kb of the human MHC class II region encoding a cluster of genes for antigen processing. *J. Mol. Biol.* **228**, 433–441
33. Tanaka, K. and Kasahara, M. (1998) The MHC class I ligand-generating system: roles of immunoproteasomes and the interferon-γ-inducible proteasome activator PA28. *Immunol. Rev.* **166**, 161–176
34. Furukawa, H., Murata, S., Yabe, T., Shimbara, N., Keicho, N., Kashiwase, K., Watanabe, K., Ishikawa, Y., Akaza, T., Tadokoro, K., Tohma, S., Inoue, T., Tokunaga, K., Yamamoto, K., Tanaka, K., and Juji, T. (1999) Splice acceptor site mutation of the transporter associated with antigen processing-1 gene in human bare lymphocyte syndrome. *J. Clin. Invest.* **103**, 755–758
35. de la Salle, H., Hanau, D., Fricker, D., Urlacher, A., Kelly, A., Salamero, J., Powis, S.H., Donato, L., Bausinger, H., Laforet, M., Jeras, M., Spehner, D., Bieber, T., Falkenrodt, A., Cazenave, J.-P., Trowsdale, J., and Tongio, M.-M. (1994) Homozygous human TAP peptide transporter mutation in HLA class I deficiency. *Science* **265**, 237–241
36. Rothman, J.E. and Wieland, F.T. (1996) Protein sorting by transport vesicles. *Science* **272**, 227–234
37. Walker, J.E., Saraste, M., Runswick, M.J., and Gay, N.J. (1982) Distantly related sequences in the alpha- and beta-subunits of ATP synthase, myosin, kinases and other ATP-requiring enzymes and a common nucleotide binding fold. *EMBO J.* **1**, 945–951
38. Saitou, N. and Nei, M. (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **4**, 406–425

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