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Membrane Topology of the ATP-binding Cassette Transporter Associated with Antigen Presentation (Tap1) Expressed in *Escherichia coli**

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The ATP-binding cassette transporters associated with antigen presentation (Tap1 and Tap2) mediate the transport of peptide fragments across the endoplasmic reticulum membrane of mammalian cells. Tap1 and Tap2 are closely related to one another and are believed to function as a heterodimer. Each protein possesses a hydrophobic domain predicted to span the membrane multiple times and a highly conserved nucleotide-binding domain. We have assessed the transmembrane topology of Tap1 by expressing a series of fusions to a reporter protein, the mature form of β-lactamase in *Escherichia coli*. From these data a topological model can be derived in which Tap1 spans the membrane eight times, with several large loops exposed in the lumen of the endoplasmic reticulum and with both the N and C termini (including the nucelotide-binding domain) residing in the cytoplasm.

Cytotoxic T lymphocytes (CTLs)[1] continually survey cells for changes in cytosolic content. Antigens from cytoplasmic proteins are presented to the CTLs at the cell surface in the form of peptide fragments complexed with major histocompatibility class I and β_2-microglobulin molecules (1). These trimeric complexes are recognized by the T cell receptor on the CTLs. To assemble this trimeric complex, the peptide fragments which are normally generated by the proteasome in the cytoplasm, must be translocated into the lumen of the endoplasmic reticulum (ER). Two proteins, Tap1 and Tap2, are required for this transport process (2, 3). Tap1 and Tap2 each consist of a hydrophobic domain predicted to span the membrane multiple times, and an ATP-binding domain, which is believed to couple the energy of ATP hydrolysis to peptide transport. Tap1 and Tap2 function as a heteromer (4–6) and are members of the ATP-binding cassette (ABC) superfamily of transporters (7).

The transmembrane domains of ABC transporters typically (although there are exceptions; see below) consist of 12 clearly defined, putative membrane-spanning segments, which could, potentially, span the lipid bilayer. For a number of ABC transporters, both prokaryotic and eukaryotic, this predicted topology has been confirmed experimentally (8–10). The N-terminal hydrophobic domains of Tap1 and Tap2 appear to differ from those of other ABC transporters in that the potential membrane-spanning segments are not clearly defined by conventional algorithms and appear to number more than 12 (for the Tap1-Tap2 complex). To clarify this situation we have analyzed the transmembrane topology of the human Tap1 protein using a genetic approach in which a reporter protein (the mature form of β-lactamase) was fused to a series of defined points along the length of the Tap1 protein. The orientation of the β-lactamase with respect to the membrane was assessed by its ability to confer ampicillin resistance when expressed in *Escherichia coli*. This approach, and the related *phoA* method, have been used, successfully, to study many other membrane proteins (11–13). The data generate a model in which Tap1 spans the membrane eight times with large extracellular (lumenal) hydrophilic loops and the N and C termini, including the nucleotide-binding domain, located in the cytoplasm. This predicted organization differs from that of many other ABC transporters, and its functional implications are considered.

EXPERIMENTAL PROCEDURES

Bacterial Strains and Plasmids—*E. coli* strain DH5αF′: *endE44 hsdR17*($r_K^-m_K^+$) *supE44 thi-1 recA1 gyrA*(Nal^r) *relA1* Δ(*lacIAYZ-argF*)U169 *deoR* (ϕ80d*lac*Δ(*lacZ*)*M15*) was used routinely. For certain studies the protease-deficient strain CH1790 (*htpR155*::Tn*10* Δ*lon ilv his sup*^{o} *strA* Δ*proC galOP*::IS1 Δ*bio*λΔ[Bam-N$^+$]) (10) was used.

Plasmid pYJ1 contains the *tap1* cDNA (14) in the general cloning vector pKG18. pYZ4 and pYZ5 are two plasmids designed to facilitate the cloning and generation of C-terminal β-lactamase fusions to eukaryotic genes.

Molecular Biology—Restriction endonucleases and DNA modifying enzymes were purchased from New England Biolabs and used according to the manufacture's instructions. The oligonucleotides used in this study are listed in Table I.

Construction of β-Lactamase Fusions—A total of 39 in-frame Tap1-β-lactamase fusions were generated. Two approaches to generating these fusions were used, a random method and a directed method.

Random fusions were generated as follows. The *tap1* gene was cloned into plasmid pYZ4 (15) under control of the inducible *E. coli lac* UV5 promoter and a bacterial ribosome binding site, generating pYZ*tap1* (Fig. 1). pYZ *tap1* was cleaved at its unique *Hin*dIII site (located near the C-terminal end of the postulated hydrophobic domain) and progressive deletions introduced into the *tap1* gene with exonuclease Bal-31 (Fig. 2*A*). These randomly truncated derivatives of *tap1* were fused to the coding sequence of the mature form of β-lactamase (Fig. 2*A*) and transformed into *E. coli* strain DH5αF′, selecting for kanamycin resistance. The resultant colonies were screened for ampicillin resistance at high cell density (see below) to determine whether they expressed an in-frame Tap1-β-lactamase fusion. Plasmids that conferred resistance were sequenced to determine the Tap1-β-lactamase fusion junctions. This approach generated 29 independent, in-frame β-lactamase fusions between amino acids 11 and 342 of Tap1 (designated p*tap*11 to p*tap*342, respectively).

Directed Tap1-β-lactamase fusions were constructed as follows. Two fusions were constructed taking advantage of unique *Pvu*II and *Hin*dIII restriction endonuclease sites within the *tap1* gene. The coding se-

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[1] The abbreviations used are: CTL, cytotoxic T lymphocyte; ER, endoplasmic reticulum; ABC, ATP-binding cassette; PCR, polymerase chain reaction.

TABLE I
List of oligonucleotides and plasmids used in this study

Oligonucleotides		
N-terminal	GTCGAAGACCCCATGGCTAGCTCTAGGTG	See Figs. 1 and 2*B*
Reverse primers:		
122	TTGGATCCCGGGGGCTCCCCATGA	See Fig. 1
40	CAGCGCGGTCCGGAGCAG	See Fig. 2
66	GGCCCAGCGGCTCAGGCC	See Fig. 2*B*
88	ACCTGCGTTTTCGCTCTT	See Fig. 2*B*
220	TGAGCCATCTTGTAGAATCCA	See Fig. 2*B*
318	CCCCAAGAGACATAGGCC	See Fig. 2*B*
323	TCCCCAGAGCATGATCC	See Fig. 2*B*
492	ACCACTGGGTGGGCAGCG	See Fig. 2*B*
748	TTCTGGAGCATCTGCAGG	See Fig. 2*B*
Plasmids		
pYJ1		Trowsdale *et al.* (14)
pYZ4		Zhang and Broome-Smith (15)
pYZ5		Zhang and Broome-Smith (15)
pYZ 5′*tap1*	pYZ4 with first 366 base pairs of *tap1*.	This study
pYZ-*tap1*	pYZ4 with all of *tap1*.	This study
pYZ-*tap15* to pYZ-*tap*748	β-Lactamase-*tap1* fusions in pYZ4. The numbers represents the fusion junctions in terms of Tap1 residue number.	This study
pYZ*tap1-Pvu*II	β-Lactamase-*tap1* fusion where junction is at the *Pvu*II site of *tap1* (equivalent to p*tap*433).	This study

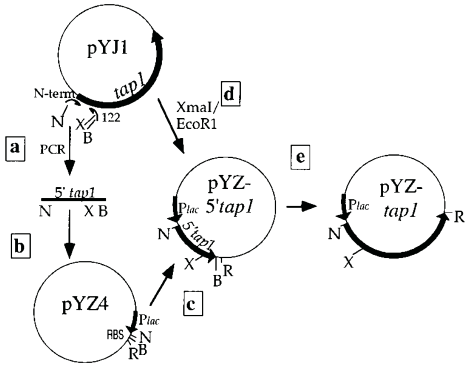

FIG. 1. **Subcloning *tap1* into the expression vector pYZ4.** The cloning was performed in two stages. First, a short DNA fragment from the 5′ end of the *tap1* gene was amplified in a PCR using the *tap1* plasmid pYJ1 as a template, and oligonucleotides "*N-term*" and "*122*" (*reaction a*). These primers introduced an *Nco*I site at the initiating ATG codon and a *Bam*HI site immediately downstream of the *Sma*I site of *tap1*. These sites were used to insert the PCR product between the *Nco*I and *Bam*HI sites of pYZ4 (*reaction c*), placing the 5′ end of *tap1* under the control of the inducible *lac* promoter (P*lac*UV5) and ribosome binding site (*RBS*). The remaining portion of the *tap1* gene was excised from pYJ1 with X*ma*I and *Eco*RI (*reaction d*) and inserted between the *Xma*I and *Eco*RI sites of pYZ-5′*tap1* generating pYZ*tap1* (*reaction e*). *N* = *Nco*I; *X* = *Xma*I; *B* = *Bam*HI; *R* = *Eco*RI.

quence of the mature form of β-lactamase, excised from pYZ5 as a *Pvu*II-*Eco*RI fragment (Fig. 1*B*), was inserted directly into the *tap1* gene of plasmid pYZ*tap1* at the unique *Pvu*II and *Hin*dIII sites (trimmed to blunt ends with mung bean nuclease). These fusions were named according to the site of the fusion junctions, p*tap*378 and p*tap433*, respectively. Eight additional directed Tap1-β-lactamase fusions were generated by the PCR (Fig. 2*B*) and named according to the position of the fusion junctions, p*tap40* to p*tap*748. The DNA sequences of all PCR-amplified DNA and the fusion junctions were determined.

Screening for Ampicillin Resistance—Plasmids containing in-frame Tap1-β-lactamase fusions were identified by their ability to confer ampicillin resistance on *E. coli* at high cell density. At high cell density all in-frame fusions, whether to intracellular or extracellular domains of Tap1, will confer ampicillin resistance due to release of β-lactamase from a proportion of the cells by lysis. Stationary-phase cultures were washed once in Luria broth (LB; Ref. 16) and resuspended in the original volume of LB. 10 μl of the cell suspension was spotted onto an LB agar plate containing 50 μg/ml kanamycin and 100 μg/ml ampicillin and incubated overnight at 37 °C. Resistance was defined as confluent cell growth within the spotted area.

Because the target for ampicillin is extracellular, isolated cells will be ampicillin-resistant only if they express β-lactamase fused to an extracellular domain. To determine whether a Tap1-β-lactamase fusion junction was intracellular or extracellular, cells were screened for ampicillin resistance at low cell density. An overnight culture of cells was washed once in LB, diluted, and 10 μl of each dilution spotted, in duplicate, onto eight LB agar plates containing 50 μg/ml kanamycin and different concentrations of ampicillin: 0, 2.5, 5, 7.5, 10, 25, 50, 100, or 200 μg/ml. Several dilutions (10^{-5}, 10^{-6}, and 10^{-7}) were used so that one would generate separate colonies (5–15 per spot) representing isolated bacteria. Plates were incubated overnight at 30 °C or until colonies appeared on plates with no ampicillin. Incubation at 30 °C rather than 37 °C was used to avoid overgrowth of bacterial colonies, which might lead to appearance of satellite ampicillin-sensitive colonies. For each Tap1-β-lactamase fusion a maximum ampicillin resistance was assigned as the highest ampicillin concentration at which the fusion permitted growth.

Western Blot Analysis—Cells (~10^8), grown to midlog phase in LB plus 50 μg/ml kanamycin, were harvested by centrifugation and lysed in SDS sample buffer at 90 °C (16). Proteins were separated by SDS-polyacrylamide gel electrophoresis, transferred onto Hybond™-C Super transfer membranes (Amersham Corp.) and fusion proteins detected using rabbit anti-β-lactamase antibodies (5 Prime → 3 Prime, Inc., Boulder, CO) using enhanced chemiluminescence (ECL; Amersham).

RESULTS

The Tap1 protein consists of an N-terminal hydrophobic domain and a C-terminal hydrophilic domain (the nucleotide-binding domain). A hydrophobicity plot of Tap1 predicts 10 clusters of hydrophobic amino acids that are of sufficient length to span the membrane (labeled as *A* to *J* in Fig. 3). To assess which of these segments actually traverse the membrane, and hence the transmembrane topology of Tap1, fusions between defined points in Tap1 and the mature form of β-lactamase were generated. When expressed in *E. coli*, β-lactamase acts as reporter of transmembrane topology. β-Lactamase breaks down ampicillin, an antibiotic whose target is extracellular (*i.e.* periplasmic in *E. coli*). If β-lactamase is fused to a point in Tap1 which is periplasmic (equivalent to the lumen of the ER), ampicillin is hydrolyzed and the cells are ampicillin resistant. If β-lactamase is fused to a point in Tap1 which is intracellular (cytoplasmic), cells are ampicillin-sensitive.

Twenty nine in-frame Tap1-β-lactamase fusions within the transmembrane domain of Tap1 were generated by random approaches. A directed approach was then taken to generate an additional 10 fusions to defined points in the sequence to ensure complete coverage of the entire transmembrane domain (see "Experimental Procedures"). At least one fusion was generated to each of the hydrophilic loops separating the 10 predicted transmembrane segments of Tap1. The precise fusion junctions, and their relation to the predicted transmembrane

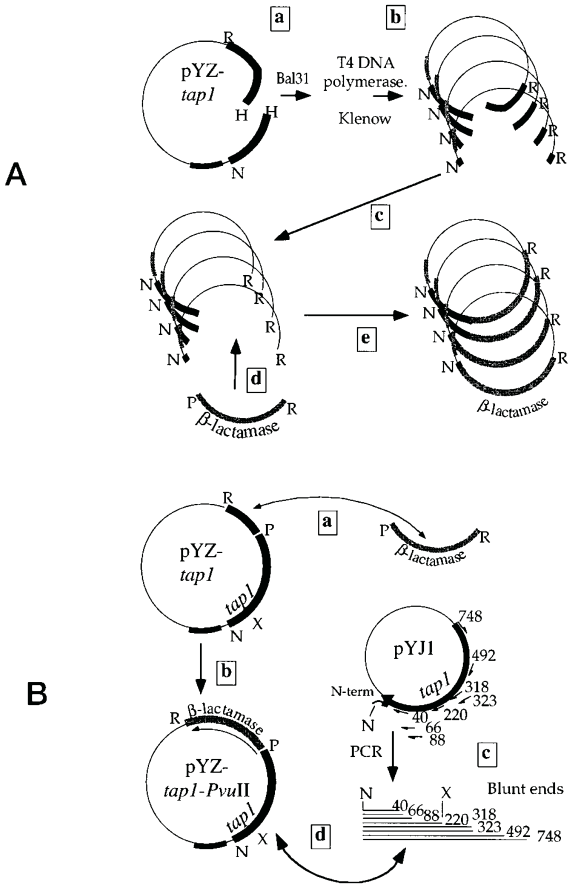

FIG. 2. **Fusion of the *blaM* gene, encoding β-lactamase, to *tap1*.** *A*, random fusions. The endonuclease Bal-31 was used to generate progressive deletions from the unique *Hin*dIII site of *tap1*. Plasmid pYZ-*tap1* was digested with *Hin*dIII and treated with Bal-31 at room temperature. Samples were removed every 2 min and the reaction stopped by addition of EGTA and transfer to 0 °C (*reaction a*). Overhanging DNA ends were filled in with the Klenow fragment of DNA polymerase and T4 DNA polymerase (*reaction b*). The remaining 3′ sequences of *tap1* were removed by digestion with *Eco*RI (*reaction c*), and the coding sequence of the mature form of β-lactamase was inserted as a *Pvu*II-*Eco*RI fragment (*reaction e*). The resultant population of *tap1-blaM* fusions was transformed into DH5αF′ selecting for kanamycin resistance. The DNA sequences of the fusion junctions were determined, identifying the amino acid residue of Tap1 to which β-lactamase was fused. *B*, directed fusions. A derivative of pYZ-*tap1* was constructed as a parental plasmid from which all the directed fusions were constructed. This was done by inserting the coding sequence of the mature form of β-lactamase into pYZ-*tap1* as a *Pvu*II to *Eco*RI fragment, replacing the 3′ region of the *tap1* gene. The resultant plasmid was designated pYZ*tap1-Pvu*II. *Eco*RI-*Xma*I or *Eco*RI-*Nco*I fragments of pYZ*tap1-Pvu*II were then replaced (*reaction d*) with one of eight different PCR products amplified using VENT DNA polymerase (*NEB*) and *tap1* (in pJY1) as a template (*reaction b*). These PCR products contained the coding sequence of *tap1* from the *Nco*I or *Xma*I sites up to one of eight specific residues: alanine 40, leucine 66, glycines 88, 318, 323, and 492, serine 220, and the C-terminal glutamate. The priming oligonucleotides were 122 and one of the following; 40, 66, 88, 318, 323, 492, 220, or 748 (*reaction c*). All the plasmids were transformed into *E. coli* strain DH5αF′, selecting for kanamycin resistance. All amplified regions and fusion junctions were sequenced to ensure no mutations had arisen.

segments, are shown in Fig. 3.

To assess the cellular location of the Tap1-β-lactamase fusion junctions, the maximum ampicillin resistance conferred by each of the fusions was assessed (Table II). Those fusions, which conferred resistance to ampicillin when plated at low density, were considered to be to fusions to an extracellular portion of Tap1 (equivalent to the ER lumen). The absolute level of ampicillin resistance differed considerably between fusions due to differences in levels of protein synthesis, stability, and/or folding. Nevertheless, any level of resistance implies an extracellular location. Those fusions, which conferred no ampicillin resistance when cells were plated at low density, were considered to place β-lactamase in a cytoplasmic location. To exclude the possibility that such fusions failed to confer ampicillin resistance because no fusion protein was made, rather than because the fusion junction was intracellular, two tests were performed. First, their ability to confer ampicillin resistance at high cell density was assessed. If β-lactamase is synthesized but remains intracellular some cells lyse, releasing β-lactamase to hydrolyze ampicillin, which allows neighboring cells to grow when cells are plated at high density. All the fusions conferred resistance at high cell density, indicating that they do indeed express Tap1-β-lactamase fusion proteins. Second, the production of β-lactamase was examined by Western blotting (Fig. 4). All fusions were shown to produce β-lactamase fusion proteins, although the predicted full-length fusions could not always be detected due to proteolysis. Transferring selected fusions into a protease-deficient strain, CH1790, showed no difference in protein degradation (data not shown). Although degradation meant that full-length protein could not be detected, the full-length fusion protein must initially be synthesized in order for ampicillin resistance to be detected (as it was for all fusions). Furthermore, degradation cannot generate false positives (*i.e.* cannot indicate a fusion is to an extracellular segment when it is not) because by definition, the high level ampicillin resistance conferred by extracellular Tap1-β-lactamase fusions demands that the β-lactamase moiety is synthesized and translocated.

Based on the above data the transmembrane topology of Tap1 expressed in *E. coli* can be determined. The predicted hydrophobic amino acids clusters (*A* to *J* in Fig. 3) were considered to be actual membrane-spanning segments when fusions upstream and downstream of the hydrophobic amino acid clusters located β-lactamase on opposite sides of the membrane. For example hydrophobic cluster B was designated as a membrane-spanning segment because an upstream β-lactamase fusion (to amino acid 40) was periplasmic, while a downstream β-lactamase fusion (to amino acid 66) was cytoplasmic. On this analysis, only 8 of the 10 predicted membrane-spanning segments traverse the membrane. These are indicated in Fig. 3. Clusters *D* and *E* do not appear to span the membrane. Some β-lactamase fusions were within potential membrane-spanning segments. These fusions conferred levels of ampicillin resistance consistent with the location of the preceding hydrophilic loop, presumably because the residual portion of the membrane-spanning segment present in the fusion was not sufficiently long to span the entire membrane or because topogenic information was present downstream of the actual membrane-spanning segment. For example, the fusion to amino acid 210 is located extracellularly, since it does not contain all the information required to transfer membrane-spanning segment F across the membrane: the downstream fusion, to amino acid 220, contains all the required topogenic elements and is located at the cytoplasmic face of the membrane. This additional topogenic information is presumably outside the hydrophobic membrane-spanning segments.

Fig. 5 (*B* and *D*) shows the topological model generated for Tap1 derived from these data. Tap1 expressed in *E. coli* spans the membrane eight times with several large extracellular loops.

DISCUSSION

Tap1 and Tap2 are related proteins which together form the Tap peptide transporter of the endoplasmic reticulum required for Class I-mediated antigen presentation. The transmem-

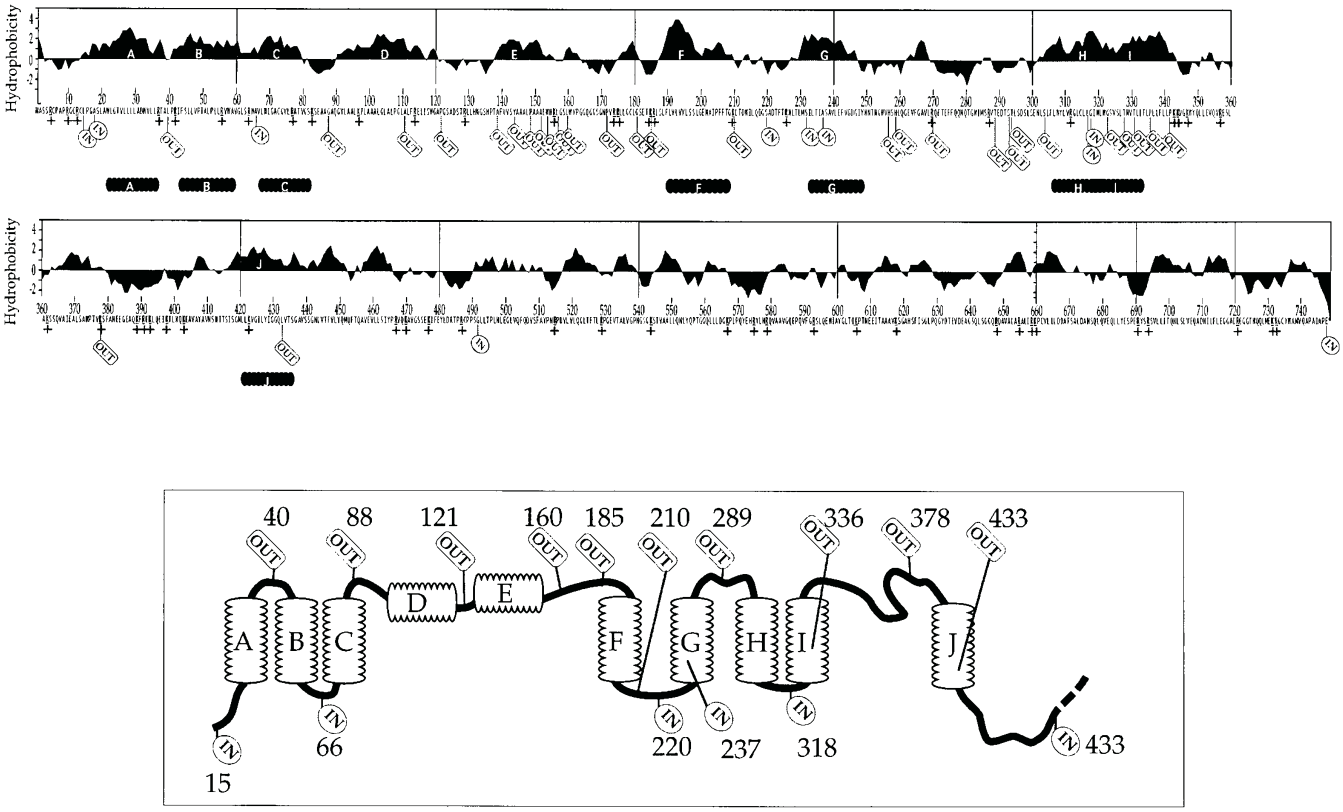

FIG. 3. **The Tap1 protein.** In the *top two panels* the amino acid sequence of Tap1 is shown together with its hydrophobicity profile generated by the MacVector program (IBI) using the Kyte and Doolittle algorithm (34) and a window size of 7. Clusters of hydrophobic sequence, which are potential membrane-spanning segments, are marked *A–J*. Arginine and lysine residues are marked + to indicate the positive charges. The sites of each β-lactamase fusion are indicated by flags marked "*In*" (cytoplasm) or "*Out*" (extracellular; corresponding to the ER lumen) according to the data presented in Table II. Below the amino acid sequence, *black blocks* indicate the experimentally determined membrane-spanning segments. The *bottom panel* shows a schematic of the proposed topology, with selected fusion locations indicated with flags and the amino acid residue number to which the fusion was made.

TABLE II
Ampicillin resistance of Tap1-β-lactamase fusions

The maximum ampicillin resistance (MAR) is given for each Tap1-β-lactamase fusion (center column) next to the fusion residue number. Where the MAR was greater than 0, the location of the fusion junction is designated Out (left column), indicating that β-lactamase resides in the periplasm (equivalent to ER lumen). Fusions which had a MAR of 0 were designated as In (*i.e.* cytoplasmic). Assays were done in duplicate and, with the exception of clone 342, produced the same MAR.

Fusion to residue no.	MAR	Location	Fusion to residue no.	MAR	Location	Fusion to residue no.	MAR	Location
	μg/ml			*μg/ml*			*μg/ml*	
15	0	In	160	25	Out	294	10	Out
18	0	In	172	100	Out	304	10	Out
40	50	Out	181	10	Out	317	0	In
66	0	In	185	10	Out	318	0	In
88	7.5	Out	210	200	Out	323	10	Out
111	200	Out	220	0	In	328	10	Out
121	10	Out	232	0	In	331	100	Out
139	25	Out	237	0	In	336	50	Out
144	10	Out	257	25	Out	342	100/50	Out
149	200	Out	259	25	Out	378	7.5	Out
152	200	Out	270	7.5	Out	433	5	Out
155	50	Out	289	25	Out	492	0	In
157	10	Out	293	10	Out	748	0	In

brane topology of Tap1 predicted from its primary sequence is, on first inspection, different from that of many other ABC transporters. To clarify the transmembrane topology of Tap1 we used an experimental approach, the toplogy reporter-protein system, which has been used extensively to monitor the transmembrane topology of membrane proteins (12), including many from eukaryotic cells (15, 17–22). Although this approach requires heterologous expression in *E. coli*, this species has previously been used to express functional eukaryotic transport proteins (23, 24), and several eukaryotic polytopic membrane proteins have been shown to fold correctly in the *E. coli* membrane (19, 22). However, it is possible that the topology of Tap1 expressed in *E. coli* differs from that in mammalian membraanes.

A topological map of Tap1 was generated, with eight membrane-spanning segments and both the N and C termini located in the cytoplasm. This places the nucleotide-binding domain in the cytoplasm, in agreement with the location of this domain

predicted by *in situ* antibody labeling experiments (5). The distribution of positive charges around the first membrane-spanning segment (three arginines preceding it and two succeeding it before the next membrane-spanning segment see Fig. 3) is consistent with the positive inside rule (25), while the distribution of positive charges around subsequent membrane-spanning segments is less adherent to this rule as is the case with other eukaryotic polytopic membrane proteins (26).

Although the β-lactamase and the related *phoA* methods for mapping transmembrane topology have been informative for membrane proteins, they involve fusing truncated versions of Tap1 to β-lactamase and any model generated must be considered within this limitation. For example, if Tap1 has C-terminal topological determinants these would be deleted in fusion proteins and may influence the folding observed. Nevertheless, this has not proved a problem in determining the folding of other polytopic membrane proteins using this method where data have been confirmed by other, biochemical approaches (9, 17, 27). The topological model presented here is consistent with other available data. Perhaps significantly, the topology of Tap1 is very similar to that determined for the MalF protein, an *E. coli* ABC transporter for maltose. MalF has eight membrane-spanning segments arranged along the polypeptide in a similar 3:2:2:1 order (Fig. 5) with similar large extracellular (lumenal) loops (8).

The transmembrane topology of Tap differs from the paradigm for ABC transporters, although several other exceptions have been reported (*e.g.* Refs. 8, 28, and 29). More importantly, the model places several large loops in the lumen of the endoplasmic reticulum. This is unusual for ABC transporters, where the large loops are generally cytoplasmic, but may reflect the fact the Tap interacts with the major histocompatibility class I molecule in the ER lumen (30–33). One of these loops contains two hydrophobic segments (clusters D and E), which were initially predicted to span the membrane. However since the experimental data suggest they do not traverse the membrane they may associate with the lumenal face of the membrane or be buried within the tertiary or quaternary structure of a protein complex in the ER.

Tap1 and Tap2 are closely related in primary sequence. However, Tap2 is slightly shorter than Tap1 (by 40 amino acids). Both *tap1* and *tap2* genes are organized in 11 exons. Comparison of the length of coding sequence within each exon shows that difference in length between the hydrophobic domains of the two proteins is mainly due to differences within the first exon. An optimal alignment of the Tap1 and Tap2 sequences (Fig. 6) indicates that hydrophobic cluster E is absent in Tap2 and that there is also considerable divergence in the region around and including hydrophobic cluster D. These

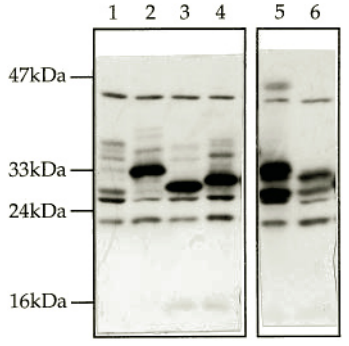

FIG. 4. **Western blot analysis of Tap1-β-lactamase fusions.** Total proteins from cells harboring different Tap1-β-lactamase fusions were separated by SDS-polyacrylamide gel electrophoresis and detected by Western blotting using an anti-β-lactamase antibody. The Tap1-β-lactamase fusions shown are to amino acids 331 (*lane 1*), 304 (*lane 2*), 289 (*lane 3*), 294 (*lane 4*), 181 (*lane 5*), and 257 (*lane 6*). The positions of molecular weight markers are shown.

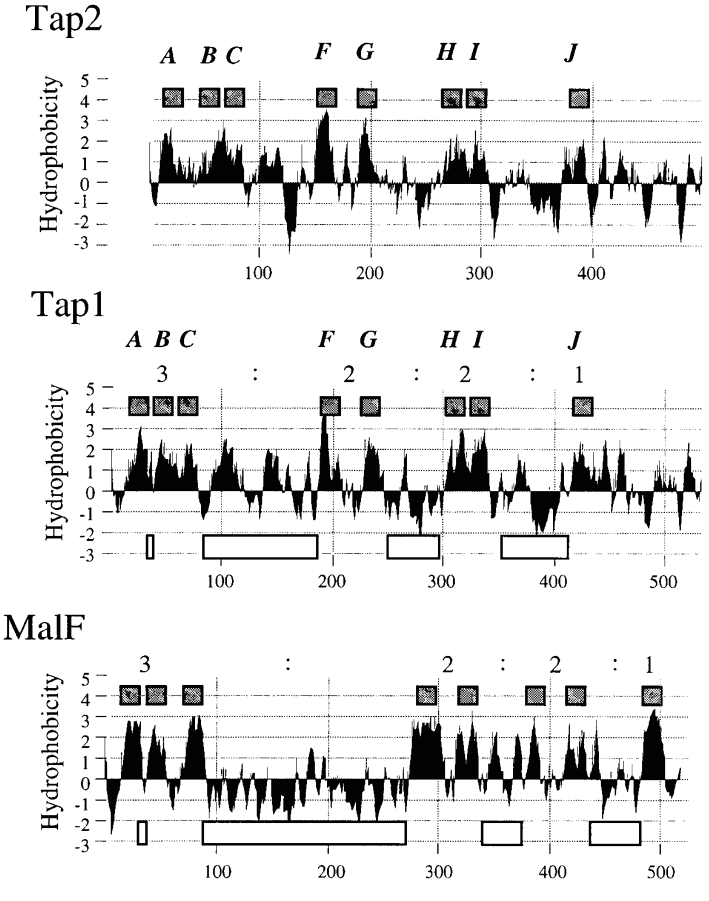

FIG. 5. **Transmembrane topology of Tap1 and a comparison with Tap2 and MalF.** The hydrophobicity profiles of the hydrophobic domain of Tap1, Tap2, and MalF are shown. The positions of the membrane-spanning segments, which have been determined experimentally, are indicated by *shaded blocks* above the hydrophobicity profiles (except in the case of Tap2, where no data are available). The Tap1 data are from this paper; the MalF data are from Froshauer *et al.* (8). *Numbers* above these *blocks* highlight the "*3:2:2:1*" arrangement of the membrane-spanning segments in the two proteins. *White blocks* below the profile indicate the position of extracellular (ER lumen for Tap1; periplasm for MalF) loops. Tap2 is shorter than Tap1, and the two proteins are aligned according to resemblance in hydrophobicity profile and arrangement of exons (see Fig. 6). The predicted membrane-spanning segments *A–J* are indicated for Tap1. The experimental data in this paper suggest that *D* and *E* do not actually span the membrane. Based on sequence alignments (Fig. 6) the equivalents of these membrane-spanning segments in Tap2 are indicated.

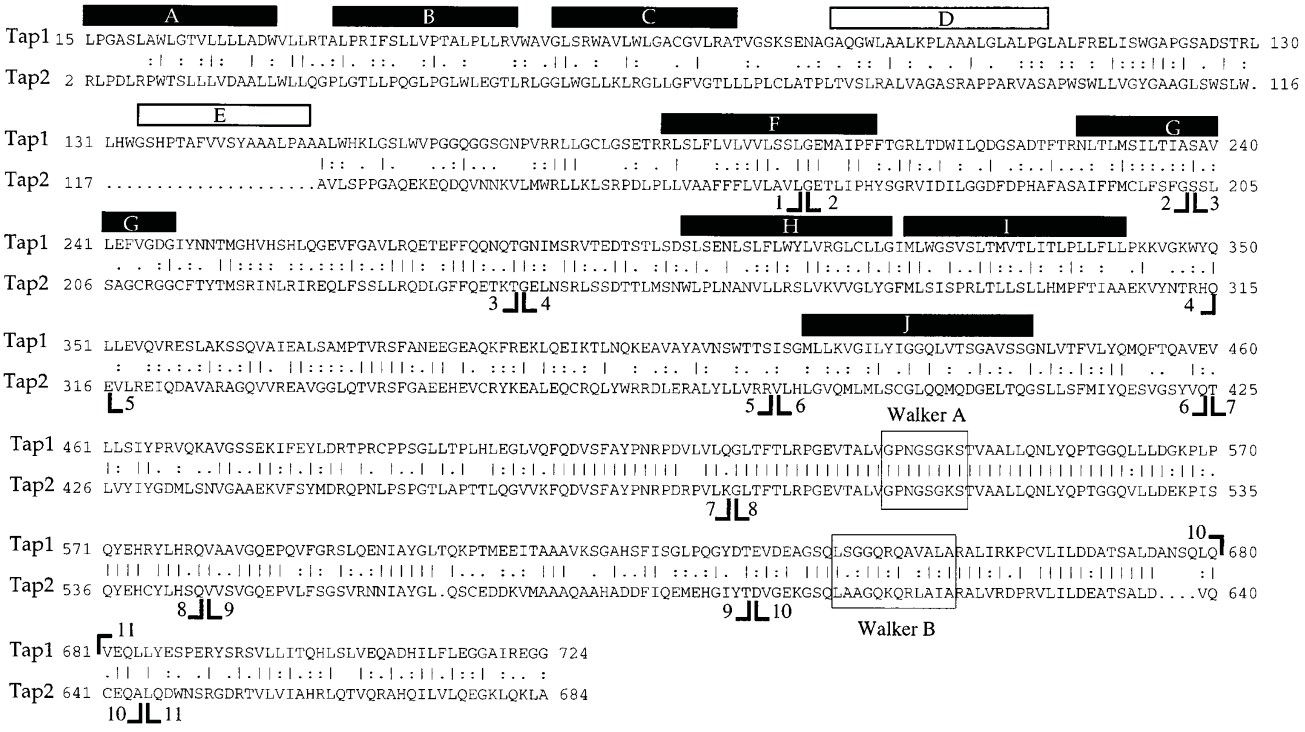

FIG. 6. **Sequence alignments of Tap1 with Tap2.** Tap1 and Tap2 were aligned using the Bestfit program of the Wisconsin University Genetics Computer Group (WUGCG) package version 8.1 with a gap weight of 10 and length weight of 0.1 (percent identity was 36.6 and similarity 58.5). *Black boxes* above the alignment indicate the hydrophobic clusters of Tap1 shown experimentally in this paper to span the membrane. *Open boxes* above the alignment indicate the predicted membrane-spanning segments, which on the basis of data in this study appear not to span the membrane. The nucleotide binding motifs Walker A and B (35), which form part of the ABC domain, are indicated for orientation. The boundaries of sequences encoded by different exons are indicated below the protein sequence. In this alignment these boundaries are identical for Tap1 and Tap2 with the exception of the boundary between exons 10 and 11.

are the two hydrophobic clusters of Tap1 which do not appear to span the membrane. Thus, it seems likely that Tap2 has the same transmembrane topology as Tap1 but that the large lumenal loop containing hydrophobic clusters D and E is much reduced in size. As Tap1, but not Tap2, interacts with the major histocompatibility class I molecule in the ER (30, 32, 33), it is tempting to speculate that this large hydrophobic loop of Tap1 plays a role in this interaction. The topology for Tap1 determined here provides a working model to facilitate further structure-function analysis.

Acknowledgments—We are grateful to Alain Townsend and John Trowsdale for the *tap1* cDNA and Tim Eilliot, Sebastian Springer, Kenny Linton, and Jenny Broome-Smith for helpful discussions.

Note Added in Proof—Since submitting this work, studies have appeared indicating the peptide binding site of Tap is in a region that our model would predict to be extracellular (*i.e.* in the ER lumen) (Momberg, F., Armandola, E. A., Post, M., and Hammerling, G. J. (1996) *J. Immunol.* **156,** 1756–1763; Nijenhuis, M., and Hammerling, G. J. (1996) *J. Immunol.* **157,** 5467–5477). As the peptide binding site is expected to be cytoplasmic, the topology of Tap1 expressed in *E. coli* is not fully consistent with these data. This remains to be resolved.

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