Biogenesis of Functional Antigenic Peptide Transporter TAP Requires Assembly of Pre-existing TAP1 with Newly Synthesized TAP2*

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The transporter associated with antigen processing (TAP) is essential for the delivery of antigenic peptides from the cytosol into the endoplasmic reticulum (ER), where they are loaded onto major histocompatibility complex class I molecules. TAP is a heterodimeric transmembrane protein that comprises the homologous subunits TAP1 and TAP2. As for many other oligomeric protein complexes, which are synthesized in the ER, the process of subunit assembly is essential for TAP to attain a native functional state. Here, we have analyzed the individual requirements of TAP1 and TAP2 for the formation of a functional TAP complex. Unlike TAP1, TAP2 is very unstable when expressed in isolation. We show that heterodimerization of TAP subunits is required for maintaining a stable level of TAP. By using an in vitro expression system we demonstrate that the biogenesis of functional TAP depends on the assembly of pre-existing TAP1 with newly synthesized TAP2, but not vice versa. The pore forming core transmembrane domain (core TMD) of pre-existing TAP2 is necessary and sufficient to allow functional complex formation with pre-existing TAP1. We propose that the observed assembly mechanism of TAP protects newly synthesized TAP2 from rapid degradation and controls the number of transport active transporter molecules. Our findings open up new possibilities to investigate functional and structural properties of TAP and provide a powerful model system to address the biosynthetic assembly of oligomeric transmembrane proteins in the ER.

The antigen processing machinery plays an important role in the cellular immune response of vertebrates for the identification of infected or malignantly transformed cells. Peptides derived from proteasomal degradation of intracellular proteins are translocated via the transporter associated with antigen processing (TAP) into the ER lumen and loaded onto major histocompatibility complex class I molecules. Presentation of “nonself” peptides at the cell surface to cytotoxic T-cells triggers the elimination of the infected or transformed cell. Lack of TAP expression e.g. in tumor tissue is associated with a dramatic loss of surface major histocompatibility complex class I and consequently promotes strongly the evasion of malignant cells from a proper cellular immune response. The ATP-binding cassette (ABC) transporter TAP is a heterodimer that comprises the two homologous subunits, TAP1 and TAP2, each of which consists of a hydrophobic transmembrane domain (TMD) and a hydrophilic, highly conserved cytoplasmic nucleotide-binding domain (NBD). The TMDs of both TAP subunits contribute to the formation of the peptide-binding site and the translocation pore. The binding and hydrolysis of ATP is believed to power the transport process by inducing conformational changes in the NBDs. These structural changes alter the conformation of the TMDs and cause the binding and movement of peptides across the ER membrane. The TMDs of TAP1 and TAP2 have unique N-terminal domains (N-domains with three to four TM segments) in addition to six core TM segments common to most ABC transporters. Like TAP, many other proteins that are synthesized in the ER have oligomeric structures. The subunits of these protein complexes are translated independently of each other and are inserted into the membrane and/or lumen of the ER during their synthesis. The assembly of multisubunit complexes in the ER seems to be a carefully regulated process and oligomerization is thought to induce structural changes in the subunits that are necessary for the protein to attain its native and functional state. Once correctly assembled, soluble or membrane-bound protein complexes achieve structural stability and escape the ER quality control mechanisms. However, unassembled orphan subunits that do not obtain their proper quaternary structure are removed from the cells by the proteasome-mediated ER-associated degradation pathway. Although it is clear that proper subunit assembly in the ER controls expression level, intracellular localization and function of oligomeric proteins, little is known about the requirements of transmembrane proteins for the formation of functional oligomers. Here, we investigated this by analyzing the heterodimeric assembly of TAP in the ER membrane.

We demonstrate that the stability of the individual TAP subunits in vivo differs markedly and that maintenance of TAP2 expression requires the formation of a complex with TAP1. TAP2 chains expressed in isolation are degraded within minutes by a proteasome-dependent pathway, whereas TAP1 chains as well as assembled TAP molecules are highly stable in vivo. By using a cell-free transcription/translation system we show for the first time that the functional biogenesis of TAP requires the assembly of pre-existing TAP1 with newly synthesized TAP2 but not vice versa. Furthermore, in support of our previous findings in intact cells, we demonstrate that the pore forming core TMD of newly synthesized TAP2 containing TM segments 4–9 is necessary for stable TAP expression.
Biosynthetic Assembly of TAP

FIGURE 1. In vivo stability of TAP1 and TAP2 in cells expressing the transporter subunits either individually or in combination. T2 cells expressing TAP1, TAP2, or TAPwt were biosynthetically labeled for 60 min in the absence (A) or presence (B) of 250 μM ALLN and chased for 6, 60, 120, and 240 min. Lysates were immunoprecipitated with anti-TAP1 (lysatess of T2(TAP1)) or anti-TAP2 antibodies (lysatess of T2(TAP2)) and resolved on a 10% SDS gel. Quantification was performed by densitometric analysis of the corresponding autoradiograph. The plots on the right of Fig. 1, A and B, show the percentage of precipitated radiolabeled TAP1 and/or TAP2 at each time point relative to the amount of TAP chains isolated directly after the pulse.

and sufficient to allow functional assembly with fully synthesized TAP1. Based on our present findings we propose a biosynthetic assembly pathway for TAP in the ER in which pre-existing stable TAP1 polypeptides protect newly synthesized TAP2 chains from rapid intracellular degradation. Thereby they act as a structural platform that directly controls the biogenesis and level of functional transporter complexes. The findings of our studies have significant implications for understanding the early events in the antigen presentation pathway and the assembly of polytopic membrane proteins in the ER.

EXPERIMENTAL PROCEDURES

Cell Lines and Antibodies—T2 is a human TAP-deficient lymphoblastoid cell line (6). T2 cells expressing rat wild-type TAPα (7) or single rat TAP1 or TAP2 chains were cultured in Iscove’s modified Dulbecco’s medium (Invitrogen) supplemented with 1 mg/ml G418 (PAA Laboratories). D90 and 116/5 are polyclonal rabbit antisera recognizing the C termini of rat TAP1α (8) or rat TAP2α (7). Horse-radish peroxidase-conjugated antibodies were purchased from Amersham Biosciences.

Cloning of Wild-type and N-terminally Truncated TAP Chains—The 2.6- or 2.4-kb EcoRI-fragments containing full-length cDNA from rat TAP1α (9) or TAP2α (8) were cloned into the EcoRI site of the pSP64 vector (Promega). The full-length rat TAP2α cDNA in pSP64 was used as template for the QuikChange™ site-directed mutagenesis procedure (Stratagene) to generate N-terminally truncated variants of TAP2. The complementary primers (Invitrogen) 5’-CCGCAGGAGCCACATGCCACC-3’ and 5’-GGTGTGCTCATGTGGGGCGGC- ATGGTGCTGGTCTGGGCGC-3’ (TAP2∆TM1 (2–88)), 5’-CGCAGACCCACATGGGCCGCCAGG-3’ and 5’-CCTGCCGGGCCGCATGTGGGGGCTGGTCGGC-3’ (TAP2∆TM3 (2–128)), 5’-CGCAGAGCCCAACCATGGGCCAGAGAGCGTCTGGC-3’ and 5’-CGCAGAGAGGGGTGCGGC-3’ (TAP2∆TM4 (2–241)) were used to generate the different N-terminally truncated TAP variants. All pSP64-TAP2∆TM constructs were fully sequenced in both directions.

Pulse-Chase Analysis, Chemical Cross-linking, Immunoprecipitation, and Western Blotting—For pulse-chase analysis methionine- and cysteine-starved cells were labeled with 500 μCi of Promix 35S label (Amersham Biosciences) for 60 min in the presence or absence of 250 μM proteasome inhibitor ALLN (N-acetyl-leucyl-leucyl-norleucinal, Calbiochem) before lysis in TBS, 1% Triton X-100 either immediately or upon incubation in IMDM chase medium for different time points. For chemical cross-linking of TAP, T2 transfectants (106 cells of T2(TAP1) and 108 T2 cells were generated by sucrose gradient fractionation as described previously (10). To strip the microsomes off their bound ribosomes, 400 μl of the microsomal suspension (concentration of 0.4 equivalent/μl corresponding to A280 = 20) were incubated with 4 ml of

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high salt buffer (50 mM Hepes, pH 7.5, 250 mM sucrose, 1 mM dithiothreitol, 1 mM KCl, and protease inhibitor mixture (Roche Applied Science)) and then centrifuged at 100,000 g for 30 min at 4 °C. The resulting membrane pellets were resuspended in 1 ml of high salt buffer and centrifuged again. Afterward, the microsomes were resuspended at a concentration of 1 equivalent/ml (corresponding to A260 = 50) in 50 mM Hepes, pH 7.5, 250 mM sucrose, 10 mM MgCl2, 1 mM dithiothreitol, and protease inhibitor mixture. Finally, aliquots of 20 μl were snap-frozen in liquid nitrogen and stored at −80 °C.

In Vitro Expression of TAP1 and TAP2—For the in vitro transcription/translation of TAP1 and TAP2 we used the TNT SP6 quick coupled transcription/translation system (Promega). Microsomes (20 equivalents) and 1 μg of plasmid-DNA were preincubated with 0.5 μl of RNasin inhibitor (40 units/μl) for 30 min on ice. Subsequently, 40 μl of the TNT quick master mix were mixed with 0.2–0.25 μg of plasmid DNA, microsomes (0.6–1.2 equivalents), and 2 μl of diethylpyrocarbonate-water. The reaction mix was then incubated in the presence of 80 μM methionine at 30 °C for 90 min. Finally, the microsomal fractions were collected by centrifugation (100,000 × g) and washed once with PBS.

Immunodepletion Assay—After in vitro expression of TAP1 and/or TAP2 chains the microsomal membranes were washed with PBS and lysed for 30 min in 100 μl of ice-cold PBS, 1% digitonin. Subsequently, in vitro expressed or pre-existing TAP subunits were quantitatively removed from the lysates by two successive rounds of immunodepletion with protein A-Sepharose conjugated anti-TAP1 or anti-TAP2 antibodies. To control the specificity of immunodepletion and co-depletion of TAP chains, microsomal lysates were incubated with free protein A-Sepharose (Amersham Biosciences) in parallel. Finally, the lysates were analyzed in Western blots probed for TAP1 and TAP2.

Peptide Transport Assay—TAP subunits were synthesized in vitro in the presence of microsomes (3 equivalents) and 24 μM model peptide S8, which was biotin-labeled at the lysine residue (TVDNKTRYR), at 30 °C for 60 min. In control experiments microsomes from T2 and T2(TAPwt) cells were incubated in the same way but without additional expression of TAP chains in vitro. Microsomes were isolated and washed as described above. Following lysis with 20 mM Tris/HCl, pH 7.5, 500 mM NaCl, 1 mM CaCl2, 1 mM MgCl2, 0.1% Nonidet P-40, 0.1% Triton X-100 (Sigma), protease inhibitor mixture (Roche Applied Science), transported glycosylated S8-biotin peptides were isolated by a 1-h incubation with ConA-Sepharose (Pharmacia) at room temperature. After extensive washing with lysis buffer, NeutAvidin-alkaline phosphatase (Pierce) was applied to the peptides in 10 mM Tris/HCl, 150 mM NaCl, 0.1 mM ZnCl2, 0.1 mM MgCl2, pH 7.0, for 30 min at 4 °C. The ConA-Sepharose beads were extensively washed with incubation buffer and incubated with a p-nitrophenyl phosphate-containing substrate solution (Sigma). After 40-min incubation at room temperature the reaction was stopped with 300 mM NaOH. To determine the quantity of transported S8 peptides, the substrate solution was separated from the ConA-Sepharose by centrifugation and the amount of p-nitrophenol resulting from the alkaline phosphatase-catalyzed hydrolysis was measured by spectrometer at a wavelength of 405 nm.

RESULTS

Newly Synthesized TAP2 Is Rescued from Rapid Proteasome-dependent Degradation by Assembly with Stable TAP1—To get insight into the biogenesis of TAP in the ER we first analyzed the in vivo stability of rat TAP1 and rat TAP2 in human T2 cells, which lack expression of endogenous TAP, by pulse-chase experiments in the presence or absence of the proteasome inhibitor ALLN (Fig. 1, A and B). The transporter subunits were expressed either individually or in combination. In the T2 transfectant expressing both transporter subunits (T2(TAPwt)) we observed no significant degradation of TAP chains during the chase period of 240 min (Fig. 1A). A similar observation was made for T2 cells expressing solely TAP1 (T2(TAP1)). In contrast, pulse-chase experiments with cells expressing only TAP2 (T2(TAP2)) show that in isolation TAP2 chains are rapidly degraded with a half-life of about 30–50 min (Fig. 1A). The observation that
TAP2 degradation could be almost completely blocked by the proteasome inhibitor ALLN (Fig. 1B) indicates that the catalytic activity of the proteasome is involved in this cellular process. Studies with mutant TAP variants (11) and biochemical cross-linking experiments using EGS (Ref. 12 and Fig. 2A) demonstrate that TAP2, unlike TAP1, has the ability to form homodimeric complexes in intact cells. Interestingly, as can be seen from the pulse-chase analysis in Fig. 2B, the population of TAP2 homodimers in T2(TAP2) is subject to rapid intracellular degradation. This suggests that, in contrast to the heterodimeric assembly of TAP, the homodimerization of TAP2 does not confer high in vivo stability.

Taken together, our findings demonstrate that the in vivo stability of the individual TAP subunits differs markedly and that the maintenance of TAP2 expression requires the interaction with long-lived TAP1 subunits.

Pre-existing TAP1 Assembles with Newly Synthesized TAP2 but Not Vice Versa—The experiments above show that the rescue of newly synthesized TAP2 chains depends on the formation of heterodimeric TAP complexes in the ER. Therefore, we were interested to find out what kind of mechanism is involved in the assembly of the TAP heterodimer. To investigate this and to separate experimentally the process of TAP complex formation from subunit degradation, we established a cell-free in vitro transcription/translation system for the expression of TAP chains in which we used purified microsomes derived from TAP-deficient T2 cells or T2 transfectants T2(TAP1) or T2(TAP2) (11) expressing the individual TAP1 and TAP2 subunits (Fig. 3A, lanes 1–3). Three different conditions for association of wild-type TAP were analyzed in our in vitro assays. In the first experiment, we expressed both TAP chains in vitro in the presence of microsomes derived from non-transfected T2 cells (Fig. 3A, lane 4). In the second and third experiment, TAP2 or TAP1 (Fig. 3A, lanes 5 and 6) was synthesized in vitro in the presence of microsomes prepared from T2(TAP1) or T2(TAP2), respectively. Under all experimental conditions efficient incorporation of in vitro expressed TAP chains into purified microsomes was observed (Fig. 3A).

To analyze whether the formation of the TAP complex requires the assembly of newly synthesized subunits or the interaction between newly synthesized and pre-existing chains, we performed immunoprecipitation experiments from detergent-lysed microsomes that were derived from the in vitro transcription/translation experiments. As can be seen from the pulse-chase analysis in Fig. 2B, the population of TAP2 homodimers in T2(TAP2) is subject to rapid intracellular degradation. This suggests that, in contrast to the heterodimeric assembly of TAP, the homodimerization of TAP2 does not confer high in vivo stability.
be seen from Fig. 3B, anti-TAP2 immunoprecipitation showed co-isolation of TAP1 when both TAP chains were expressed in vitro at the same time in the presence of purified T2 microsomes. Interestingly, stable complex formation of the transporter could be also observed when TAP2 was translated in vitro in the presence of microsomes from T2(TAP1), whereas no heterodimerization of TAP was detected when TAP1 was expressed in vitro in the presence of microsomes from T2(TAP2). This suggests that TAP assembly requires newly synthesized TAP2 but not TAP1. To validate these findings and to determine the efficiencies of complex formation, we performed immunodepletion experiments with microsomal lysates from T2, T2(TAP1), and T2(TAP2) after expression of TAP1 and/or TAP2 in vitro. For these studies we used anti-TAP antisera that specifically remove TAP1 or TAP2 chains from detergent-lysed microsomes (Fig. 4A). As a control we used microsomal lysates from T2(TAPwt) (lane 1). Lysates, incubated with free protein A-Sepharose (control) or immunodepleted with protein A-Sepharose-conjugated anti-TAP2 (ID:TAP2), were analyzed in Western blots probed for TAP1 or TAP2 and quantified by densitometric scanning. The obtained peak integrals of TAP1 and TAP2 signals were plotted in arbitrary units.

In conclusion, our findings demonstrate that TAP1 and TAP2 differ in their structural and temporal requirements for heterodimeric complex formation. We propose that efficient heterodimerization of TAP molecules in the ER requires the interaction of pre-existing TAP1 with newly synthesized TAP2.

Newly Synthesized TAP2 Requires an Intact Core TMD for Stable and Functional Complex Formation with Pre-existing TAP1—It has been previously shown that the six TM segments of the core TMD of TAP2 are sufficient for the functional heterodimeric assembly with TAP1 in intact cells (5, 13). To dissect the heterodimeric assembly of TAP in a
more detailed way, we constructed a series of N-terminally truncated TAP2 chains in which we deleted the transmembrane helices TM1 to TM5 by progressively removing the N-terminal sequences (Fig. 5A). 2ΔTM1, 2ΔTM2, 2ΔTM3, 2ΔTM4, and 2ΔTM5 indicate five different truncated TAP2 chains lacking residues 2–42, 2–88, 2–128, 2–185, and 2–241, respectively (Fig. 5A). After expression of the different TAP2 variants in vitro in the presence of microsomes from T2(TAP1), we tested the complex formation between pre-existing TAP1 and newly synthesized TAP2 variants by immunodepletion experiments. As shown in Fig. 5B, all truncated TAP2 chains were efficiently expressed in vitro and integrated into microsomes prepared from T2(TAP1) to a comparable extent. However, only 2ΔTM1, 2ΔTM2 and 2ΔTM3 showed efficient complex formation with TAP1, whereas assembly was reduced or not observed for variants 2ΔTM4 and 2ΔTM5. This indicates that the structural integrity of the core TMD of TAP2, but not the presence of its N-domain, is necessary to allow efficient assembly with pre-existing TAP1.

To explore whether in vitro expressed wild-type TAP2 and the N-domain-deprived variant 2ΔTM3 can participate in a functional heterodimeric peptide transporter, we measured transport activity of TAP complexes produced from T2(TAP1)-derived microsomes by transport assays with biotinylated model peptides. Purified microsomes from T2(TAPwt) and non-transfected T2 cells were used as additional controls. As can be seen from Fig. 6, TAP1/TAP2 and TAP1/2ΔTM3 complexes, which were generated by expression of TAP2 variants in vitro in the presence of microsomes derived from T2(TAP1), showed a 50–60% transport activity when compared with wild-type TAP in T2(TAPwt) microsomes. Thus, a significant amount of in vitro synthesized TAP molecules reaches a transport-active conformation. This strongly suggests that assembly between in vitro synthesized TAP2 and pre-existing TAP1 reflects a functional TAP complex formation in intact cells.

In further transport assays, we used T2(TAP1)-derived microsomes containing in vitro expressed 2ΔTM4 and 2ΔTM5. In both cases very little or no peptide transport activity was measured (Fig. 6). Together with the results depicted in Fig. 5, this suggests that in contrast to the first N-terminal TM segments (N-domain), the TM segments 4 and 5 in the core TMD of newly synthesized TAP2 are part of the contact region that allows stable and functional interaction with pre-existing TAP1.

DISCUSSION

Although TAP is one of the most intensely studied ABC transporters and constitutes a suitable model for many other members of the ABC transporter family, virtually nothing is known about its biogenesis in the ER. The pulse-chase experiments in Fig. 1 show that the stability of non-assembled TAP1 and TAP2 in vivo differs markedly. TAP2 expressed in isolation is rapidly degraded by a proteasome-dependent pathway with a half-life of 40 min while no significant degradation of TAP1 is seen over 240 min. High protein stability is also observed for assembled TAP subunits in T2-cells co-expressing TAP1 and TAP2. Although TAP2 can form homodimers when expressed in isolation, this is not sufficient to prevent degradation of the protein (Fig. 2B). This suggests that a stable protein conformation of TAP2, which protects the subunit from rapid degradation, requires physical association with TAP1. However, the experimental design of pulse-chase experiments on protein subunits, like TAP1 and TAP2, with different in vivo stability, makes it difficult to determine whether newly synthesized subunits pair with new or pre-existing subunits. Thus, we sought to analyze the process of TAP complex assembly in a cell-free in vitro transcription/translation system. Our experiments in Figs. 3–6 show for the first time that a highly efficient and functional biogenesis of TAP heterodimers critically depends on the assembly of pre-existing fully synthesized TAP1 with newly synthesized TAP2.

The role of TAP1 as an assembly platform for newly synthesized unstable TAP2 would explain the puzzling and so far poorly understood observation that the expression level of TAP1 determines the number of functional heterodimeric TAP molecules in the ER (14, 15). A modulation of the TAP1 expression under certain pathological conditions (15) may have significant implications in the context of viral infections or cancer. As suggested by a dramatic down-regulation of TAP in tumors of diverse tissue origin, sufficient levels of the TAP transporter are believed to be essential for a normal cytotoxic T-lymphocyte-mediated anti-cancer immune response and an efficient clearance of malignantly transformed cells (2). The down-regulation of TAP is much more pronounced in metastatic than in primary carcinoma lesions suggesting that a reduction in the levels of functional transporter may be directly associated with the progression of disease (2).

Most interestingly, depending on the assembly state of TAP1, different subregions of its TMD are used for tapasin binding. Heterodimeric assembly of the TAP subunits is accompanied by a displacement of tapasin from the core TMD to the N-domain in TAP1, whereas TAP2 interacts with tapasin exclusively via the N-domain (5). Hence, regarding the properties of the TAP1/2ΔTM3 transporter, tapasin binding to TAP2 is clearly not essential for efficient assembly (Fig. 5B) or transport function (Fig. 6; (5)) of TAP. However, since tapasin is required for the accumulation of normal TAP1 protein levels (1), it may act as a chaperone that prevents premature homodimerization and rapid degradation of TAP1 chains by stabilizing the core TMD for interaction with newly synthesized TAP2 chains. In view of this and our present data, we propose that the biosynthetic assembly of TAP is controlled in a way that the level of TAP1 dynamically regulates the escape of newly synthesized TAP2 from ER-associated degradation and thereby determines the amount of functional transporters in the ER. We note that this is somewhat reminiscent of the oligomerization-dependent stabilization of the α-subunit in P-type Na,K-ATPases (16, 17). Consequently, this type of mechanism is likely to have a general significance for the co-ordinate regulation of assembly of multisubunit transmembrane proteins synthesized in the ER.

As mentioned above, TAP belongs to the family of the ABC transporters (18). A distinguishing feature shared by the members of this large protein family is their modular structure. Each transporter molecule consists of four domains, including two TMDs and two NBDs, which are encoded either as a single polypeptide (tandem transporter), as two half-molecules (like TAP) or as three or four separate polypeptides (18). Different studies have demonstrated that the N- and C-terminal halves of tandem ABC transporters, like MDR1 and CFTR, utilize
different biogenesis pathways (19, 20). Thus, despite their conserved topology, corresponding TM segments within the N- and C-terminal TMDs encode dissimilar topogenic information and use different translocation mechanisms to acquire their final topology (19, 20). A recent publication showed that co- and post-translational steps as well as interactions between domains are required for proper folding of CFTR (21). The N-terminal half of this tandem transporter is thought to fold in a co-translational manner whereas the domains of the C-terminal half fold post-translationally (21). Furthermore, it seems that in CFTR the correct post-translational folding of the C-terminal domains depends strictly on the proper co-translational folding/assembly of the N-terminal domains (21). Our data suggest that the biosynthetic assembly of the TAP-TMDs could be similar to that described for CFTR. In this scenario, the TAP1-TMD would analogous to the N-terminal half of CFTR mediate stable domain folding and correct membrane insertion of the TM segments of newly synthesized TAP2. Hence, our study suggests that tandem and half-size transporters, despite having a radically different genetic structure, may share a conserved assembly pathway.

Cross-link experiments indicate that TAP2 expressed in isolation, in contrast to TAP1, tends to form homodimers (Fig. 2 and Ref. 12). In view of the distinct stability of the two TAP chains in vivo, this could suggest that TAP2 subunits are more sensitive to non-productive side reactions during the folding and assembly pathway than are TAP1 chains. The finding that pre-existing TAP2 subunits from microsomes of the transfectant T2(TAP2) do not interact with newly synthesized TAP1 suggests that homodimeric TAP2 complexes (Fig. 2) are “dead-end products” that largely prevent the normal formation of heterodimeric TAP molecules. Our own studies revealed that TAP2 homodimerization occurs in cell lines expressing single TAP2 but not in cells expressing both peptide transporter subunits (data not shown). This is in line with the observation that TAP2 chains interact more weakly with each other than with TAP1 subunits (12). In view of this, it is reasonable to assume that in the presence of both transporter subunits the process of functional TAP1/TAP2 assembly is highly favored over the non-functional homodimerization of TAP2.

As truncation mutants of TAP1 and TAP2 have proved useful to examine the structural organization and function of TAP chains (5, 13), we created N-terminally truncated TAP2 variants to analyze the requirements for newly synthesized TAP2 to form a complex with pre-existing TAP1. TAP chains have unique N-terminal domains (N-domains) in addition to six core TM segments (13). We and others could previously show that the presence of these core TMDs is sufficient (5, 13) and necessary (22) for functional TAP assembly. Our findings on the in vitro expressed deletion variants of TAP2 indicate that this also holds true for in vivo expressed TAP complexes. In the case of variant 2DTM4, in which the TM segments TM1–TM4 are deleted, a 30–40% decrease in complex formation and a ≈80% reduction of transport activity was observed (Fig. 5). Similar observations were made for N-terminally truncated human TAP variants that were heterologously and transiently expressed in insect cells (22). This suggests that the TM4 segment in the core TMD of TAP2 plays a critical role in the proper arrangement and stabilization of a functional TMD interface after complex formation with TAP1. No detectable complex formation or peptide transport could be observed for the transporter variant TAP1/2DTM5 that lacks TM1–TM5 segments in the in vitro expressed TAP2 chain (Fig. 5). Since the remaining TM segments of N-terminally truncated TAP2 variants are proposed to have the same membrane topology as in full-length TAP2 (23), we conclude from our findings that the TM segments 4 and 5 in the core TMD are of critical importance for the functional assembly of newly synthesized TAP2 with pre-existing TAP1. On the sequence level, the TM segments of the core TMDs of TAP1 and TAP2 align well with the TM segments of ABC transporters for which the TMD structures have been solved, e.g. MsbA (24). The current view is that in the heterodimeric peptide transporter the assembled TMDs of TAP1 and TAP2 have grouped their two sets of six MsbA-related core TM segments (TM5–10 in TAP1 and TM4–9 in TAP2) in an antiparallel configuration (11). In agreement with the biochemical experiments on P-glycoprotein (25–28) and the structural analysis of MsbA (24), the different assembly properties of TAP2 and its N-terminal deletion variants (Figs. 5 and 6) support a structural model of TAP in which the major dimerization interface between opposing TAP subunits is formed by the second and fifth TM segment in the core TMDs of TAP2 (TM5 and TM8) and TAP1 (TM6 and TM9).

Our findings demonstrate that the in vitro expression system provides a straightforward experimental tool to analyze the biosynthetic assembly of ER-localized oligomeric polytopic transmembrane proteins. The experimental results of this study open up new possibilities to investigate the structure and function of TAP, in particular with respect to the relative orientation and function of the TM segments at the domain interface between the core TMDs of TAP1 and TAP2.

Acknowledgments—We are indebted to Drs. R. Wilson, J. C. Howard, and G. Praefcke for their helpful comments on the manuscript.

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