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Assembly and Function of the Major Histocompatibility Complex (MHC) I Peptide-loading Complex Are Conserved Across Higher Vertebrates*

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Background: The transporter associated with antigen-processing TAP is crucial for the adaptive immune response against infected cells.
Results: Avian and mammalian TAP1, TAP2, and tapasin assemble a functional translocation and peptide-loading complex (PLC).
Conclusion: Assembly of the PLC is conserved, whereas elements of antigen translocation diverged later in evolution.
Significance: This specification provides important insights in how the antigen-processing machinery adapted in different species.

Antigen presentation to cytotoxic T lymphocytes *via* major histocompatibility complex class I (MHC I) molecules depends on the heterodimeric transporter associated with antigen processing (TAP). For efficient antigen supply to MHC I molecules in the ER, TAP assembles a macromolecular peptide-loading complex (PLC) by recruiting tapasin. In evolution, TAP appeared together with effector cells of adaptive immunity at the transition from jawless to jawed vertebrates and diversified further within the jawed vertebrates. Here, we compared TAP function and interaction with tapasin of a range of species within two classes of jawed vertebrates. We found that avian and mammalian TAP1 and TAP2 form heterodimeric complexes across taxa. Moreover, the extra N-terminal domain TMD0 of mammalian TAP1 and TAP2 as well as avian TAP2 recruits tapasin. Strikingly, however, only TAP1 and TAP2 from the same taxon can form a functional heterodimeric translocation complex. These data demonstrate that the dimerization interface between TAP1 and TAP2 and the tapasin docking sites for PLC assembly are conserved in evolution, whereas elements of antigen translocation diverged later in evolution and are thus taxon specific.

As a key component of the peptide-loading complex (PLC),[2] the transporter associated with antigen processing (TAP) plays a central role in the adaptive immune response against virus infected or malignantly transformed cells (1–3). The TAP complex translocates proteasomal degradation products from the cytosol into the ER, where these peptides are loaded onto major histocompatibility complex class I (MHC I) molecules. This step is catalyzed by the MHC I specific chaperon tapasin. Peptide-MHC I complexes can dissociate from the PLC and traffic *via* the secretory pathway to the cell surface, where they present their antigenic cargo to cytotoxic T lymphocytes.

The interplay between TAP, tapasin, and MHC I is an intriguing example of coevolution during the development of the adaptive immune system in jawed vertebrates. In humans, the MHC I gene region has been disconnected from the MHC II locus, where other components of the PLC, *e.g.* TAP1, TAP2, and tapasin, are encoded. This re-arrangement has resulted in a lower recombination rate and nearly monomorphic PLC components that cooperate with many MHC I alleles (4). In humans, limited numbers of TAP allomorphs display a broad peptide specificity, whereas the six MHC I alleles are highly polymorph to present a large epitope repertoire to cytotoxic T-lymphocytes. In contrast to mammals, avian TAP genes are located within the MHC I region leading to stable haplotypes of polymorphic genes interacting with each other (4). This coevolution of TAP and MHC I molecules leads to the expression of only one pre-dominant MHC I allele, which is accompanied by several TAP allomorphs, resulting in a haplotype-specific peptide transport in birds (5).

The heterodimeric ABC transport complex TAP consists of TAP1 (ABCB2) and TAP2 (ABCB3), each harboring a transmembrane domain (TMD) followed by a cytosolic nucleotide-binding domain (NBD) (3). By a head-to-tail dimer arrangement, the two NBDs coordinate two ATP molecules at two non-equivalent nucleotide-binding sites to translate ATP binding/hydrolysis events into long-range conformational changes of the TMDs, moving peptide substrates across the ER membrane (6). The TAP complex can be dissected into a core TAP

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[2] The abbreviations used are: PLC, peptide-loading complex; ABC, ATP-binding cassette; CH, coupling helix; NBD, nucleotide-binding domain; MHC I, major histocompatibility complex class I; TAP, transporter associated with antigen processing; TM, transmembrane; TMD, transmembrane domain.

complex, which is composed of the six C-terminal transmembrane helices (TMHs) together with the NBDs of each subunit and four N-terminal TMHs, named TMD0 (7, 8). The coreTAP complex has been identified to be essential and sufficient for peptide binding and translocation (7), while the TMD0s act as autonomous interaction platforms for tapasin binding and assembly of the PLC (8–10) (Fig. 1*A*). Maximal two tapasin molecules are assembled at the TAP complex; however, one tapasin bound either to TAP1 or TAP2 is sufficient for efficient peptide loading of MHC I and antigen presentation (11, 12).

In birds, TAP1 has evolved differently than TAP2. Notably, all avian TAP1 genes sequenced so far lack a region coding for a TMD0 and are therefore structurally similar to a coreTAP1, which fails to associate with tapasin (7, 10). The allosteric crosstalk between the TMDs and the NBDs is mediated by several key elements such as the coupling helices (CH), with CH1 being crucial for antigen translocation but not for binding (13), or the Q- and X-loops, which interact in *cis* and *trans* with both TMDs (13, 14).

So far, peptide transport by the TAP complex has only been studied in a few species, namely human, mouse, rat, and chicken (5, 15–19). Here, we cloned, expressed, and analyzed the function of more than 20 heterodimeric TAP complexes across different taxa: six mammals (human, cow, pig, dog, mouse, rat: *Homo sapiens*, *Bos taurus*, *Sus scrofa, Canis familiaris*, *Mus musculus*, and *Rattus norvegicus,* respectively), and four birds (duck, quail, chicken, turkey: *Anas platyrhynchos*, *Coturnix coturnix*, *Gallus gallus,* and *Meleagris gallopavo,* respectively). We delineate that avian and mammalian TAP1 and TAP2 dimerize across taxa and that all TAP subunits containing a TMD0 recruit tapasin. In contrast, only TAP complexes originating from species of the same taxon translocate peptides.

EXPERIMENTAL PROCEDURES

Cloning and Construct Design—TAP1 and TAP2 from *C. coturnix* (Q76LI9 and Q9PWI8, respectively) and *M. gallopavo* (B1N1D9 and B1N1E0) were synthesized (Invitrogen, Darmstadt, Germany), all other genes were amplified from cDNA libraries (20) and cloned using FX cloning into pINITIAL (21) for sequencing. All genes were subcloned into the expression vectors pcDXc3YCH (TAP1) or pcDXc3CMS (TAP2). The plasmid pcDXc3YCH was generated by replacing eGFP and the streptavidin-binding peptide (SBP) with mVenus followed by a C8-tag (PRGPDRPEGIEE) and a His_{10}-tag. In pcDXc3CMS, the region coding for eGFP was exchanged by mCerulean (Fig. 1*B*). The fluorescent proteins can be cleaved by human rhinovirus 3C protease. Tapasin was PCR amplified with the primers 5′-ATATATGTCGACATGAAGTCCCTGTCTCTGCT-3′ (forward) and 5′-TATATAGGA TCCTCACTCTGCTTTCTTCTTTG-3′ (reverse), digested with SalI and BamHI, and cloned into pcDNA3.1(+).

Transfection—HEK293T cells were transfected using polyethyleneimine (Sigma-Aldrich) as transfection reagent. Three days prior to transfection, 2×10^6 cells were seeded in DMEM/10% FCS in 15-cm dishes. 30 μg of plasmid-DNA (equimolar ratio of TAP1, TAP2, and tapasin) and 90 μg of PEI were separately diluted in 700 μl of serum-free DMEM. The two solutions were mixed, incubated for 30 min at room temperature, and vortexed several times. Prior to transfection, the medium was replaced by 10 ml serum-free DMEM and the DNA-PEI mixture was added dropwise. After 6 h, the medium was replaced by 15 ml DMEM/10% FCS. Two days after-transfection, cells were harvested with a cell scraper, washed with ice-cold PBS, and stored at −80 °C until further use.

TAP1-negative BRE169 (22) and TAP2-negative STF169 cells (23) were transfected using X-tremeGENE (Roche, Penzberg, Germany). One day prior to transfection, 2.5×10^5 cells were seeded in 2 ml of DMEM/10% FCS. 2 μg plasmid-DNA and 6 μl of X-tremeGENE were separately diluted in 150 μl serum-free DMEM. Both dilutions were combined, incubated for 30 min at room temperature, and vortexed several times. Prior to transfection, the medium was replaced by 500 μl of serum-free DMEM, and the transfection mixture was added dropwise. After 4 h, the medium was replaced by 2 ml of DMEM/10% FCS. Two days after transfection, cells were harvested by trypsination, stained with antibodies, and analyzed by flow cytometry.

Flow Cytometry—Surface expression of peptide-loaded MHC I molecules was monitored with the monoclonal antibody W6/32 (BioLegend, San Diego, CA) and an APC- or PerCP-Cy5.5-coupled secondary anti-mouse antibody (BioLegend), respectively. Flow cytometry data were recorded at a FACS Canto II (BD Biosciences, Heidelberg, Germany) or an Attune (Invitrogen) and processed with FlowJo 8.8.7 (Tree Star, Inc., Ashland, OR). Cells were harvested by trypsination and washed with ice-cold PBS. All further steps were carried out on ice. The cells were resuspended in 100 μl of FACS buffer (PBS, 2% FCS) and incubated with 0.25 μg W6/32 antibody for 1 h. Excess antibody was removed by washing with FACS buffer. Cells were incubated with 0.2 μg of a rat anti-mouse antibody (APC- or PerCP-Cy5.5-coupled) in 100 μl of FACS buffer for 1 h. Finally, cells were washed and resuspended in 500 μl of FACS buffer.

Peptide Transport—TAP1-deficient BRE169 cells were transfected with single mammalian or avian TAP1 fused to mCherry, or combinations of TAP1-mCherry and TAP2-mCerulean. Mock transfections (pcDNA3.1-mCherry) were used as control. Transfected cells were semi-permeabilized with 0.25 μg/ml streptolysin-O in PBS buffer supplemented with 10 mM of $MgCl_2$. Transport of RRYQ*NSTC*^{F}L (30 nM) was started by adding ATP or ADP (10 mM each) and was performed for 15 min at 37 °C. The transport reaction was stopped with 20 mM of EDTA in PBS. Translocated peptides were quantified by fluorescence at $\lambda_{ex/em}$ = 485/520 nm.

Tandem Affinity Purification of TAP and PLC—Cell pellets were solubilized with 1% digitonin in ice-cold buffer A (50 mM Tris/HCl, 250 mM NaCl, 10% glycerol, 0.05% digitonin, pH 8.0), and incubated for 1 h in an overhead shaker. All steps were carried out at 4 °C. The suspension was centrifuged at 150,000 × *g* for 1 h. Recombinant proteins were bound to 100 μl of streptavidin-agarose beads (Pierce) for 1 h. The beads were washed two times with 1 ml of buffer A, and bound proteins were eluted with 1 ml of buffer A supplemented with 2.5 mM biotin. 10 μg of the C8 antibody were bound to 50 μl of sheep anti-mouse IgG Dynabeads (Invitrogen) for 1 h, washed twice

with 1 ml of buffer A, and incubated with the eluate of the streptavidin-agarose beads for 1 h. After washing, the immunoprecipitate was eluted with 100 μl of 2× SDS buffer for 30 min at 37 °C. Precipitated proteins were analyzed after SDS-PAGE by in-gel fluorescence and immunoblotting.

In-gel Fluorescence and Immunoblotting—TAP1-mVenus and TAP2-mCerulean were visualized by in-gel fluorescence using a Typhoon scanner (GE Healthcare, Freiburg, Germany). Briefly, protein samples were analyzed by Tris/Tricine-PAGE (8%, 120 V). TAP2-mCerulean and TAP1-mVenus were detected at $\lambda_{ex/em}$ = 457/526 nm (short-pass filter) and 488/555 nm (± 20 nm band-pass filter), respectively. For immunoblotting, the protein samples were heated to 95 °C for 10 min to unfold the GFP. Protein samples were analyzed by Tris/Tricine-PAGE (8%) and subsequent immunoblotting using the primary antibodies α-C8 (TAP1), α-myc (TAP2) (clone 4A6, Merck Millipore, Darmstadt, Germany), or α-tapasin (clone 7F6) (9). The chemiluminescence was recorded with a LumiImager (Roche) and analyzed with LumiAnalyst 3.1.

RESULTS

Expression Screening of TAP Subunits—We first compared the expression of TAP1 and TAP2 originating from representative mammals and avian species. TAP1 was supplemented with a C-terminal C3 protease cleavage site, followed by mVenus, a C8-tag, and a His_{10}-tag. TAP2 was fused to a C-terminal C3 protease cleavage site, followed by mCerulean, a myc-tag, and a streptavidin-binding peptide (Fig. 1*B*). Although we tried different sources of cDNA and different primers, we were not able to obtain a full-length clone of *A. platyrhynchos* TAP1. To analyze the expression and function of TAP2 from *A. platyrhynchos*, we expressed it together with TAP1 from *G. gallus*.

The expression of the TAP genes was examined by flow cytometry using mVenus and mCerulean as reporter. All mammalian TAP1 and TAP2 were expressed in HEK293T cells (Fig. 2*A*). Cotransfection of TAP1/TAP2 led to an increased expression of both subunits if compared with the expression of single subunits. Avian TAP1/TAP2 pairs were expressed at a higher level than the mammalian TAP subunits. Worth mentioning, the fluorescence quantum yield of mVenus is significantly higher in comparison to mCerulean. The expression of TAP1-mVenus and TAP2-mCerulean was confirmed by SDS-PAGE in-gel fluorescence and immunoblotting using C8 and myc-tag specific antibodies to compare subunits from different species (Fig. 2*B*). All constructs were expressed at the predicted molecular mass. Interestingly, the expression level correlates with the GC content of the TAP genes with avian TAPs having an average GC content of 64 ± 2%, whereas mammalian TAPs only have an average GC content of 59 ± 1%. We did not further investigate whether this effect is based on differences in transcription, translation, or protein stability.

The Assembly of TAP1, TAP2, and Tapasin Is Conserved across Mammals and Birds—The recruitment of tapasin by TAP is essential for the assembly of the peptide-loading complex. We therefore monitored the formation of TAP1/2-tapasin complexes after tandem-affinity purification of heterodimeric TAP complexes. This was achieved by transfecting HEK293T cells with either mammalian or avian TAP1/TAP2 in various

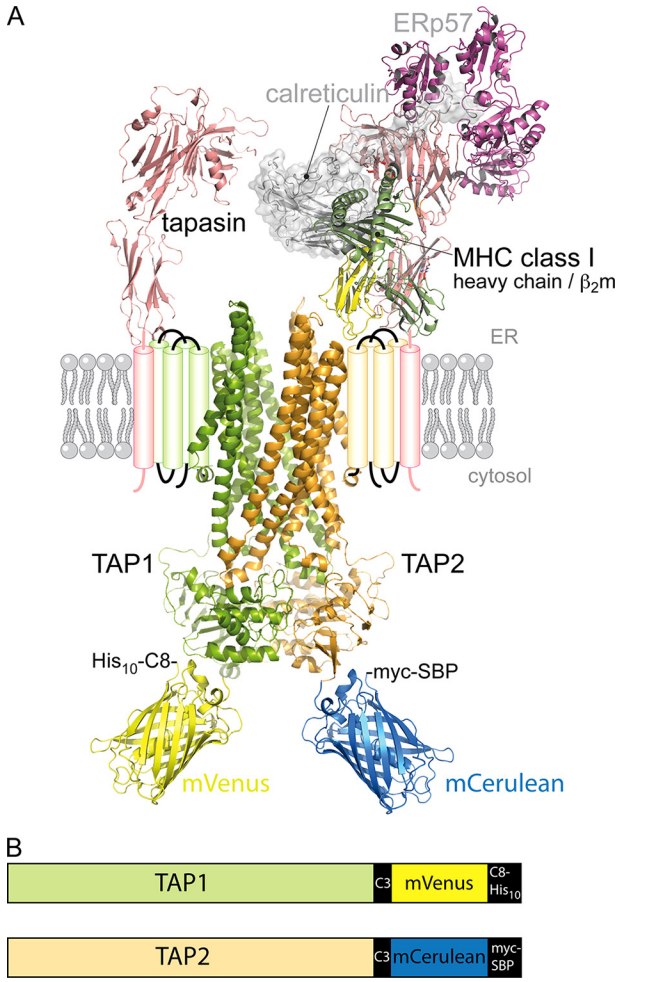

FIGURE 1. **Expression screening of TAP subunits and assembly of a PLC.** *A*, core TAP is shown as a homology model based on the x-ray structures of Sav1866 (PDB 2HYD) and an NDB dimer of rat TAP1 (PDB 2IXE) (13, 35). The interaction with tapasin (PDB 3F8U) (36) and the interaction scaffold TMD0 are indicated. *B*, TAP1 subunits are fused C-terminally to a C3 cleavage site, mVenus, a C8-tag, and a His_{10}-tag, TAP2 subunits are fused C-terminally to a C3 cleavage site, mCerulean, a myc-tag, and the streptavidin-binding peptide (SBP).

combinations with human tapasin. TAP complexes were subsequently tandem-affinity purified first *via* the streptavidin-binding tag of TAP2 and specific elution with biotin, and second *via* the C8-tag fused to TAP1. This strategy ensures that strictly heterodimeric TAP1/2 complexes are analyzed (20). The interaction with tapasin as key component for the assembly of the PLC was examined by subsequent immunoblotting. Notably, tapasin was copurified in all cases, if the TAP1/2 complex harbors at least one TMD0 (Fig. 3, *left panel*). As previously demonstrated (7), the human coreTAP complex lacking both TMD0s was not able to recruit tapasin. All mammalian TAP complexes bind tapasin and are thus able to assemble the PLC for optimal peptide loading onto MHC I molecules. Surprisingly, all avian TAP complexes can assemble chimeric PLC with subunits originating from different taxa. In these cases, the TMD0 of avian TAP2 is essential and sufficient for mediating the interaction with human tapasin, because all avian TAP1 subunits sequenced so far lack a TMD0, in analogy to human coreTAP1 (7, 8).

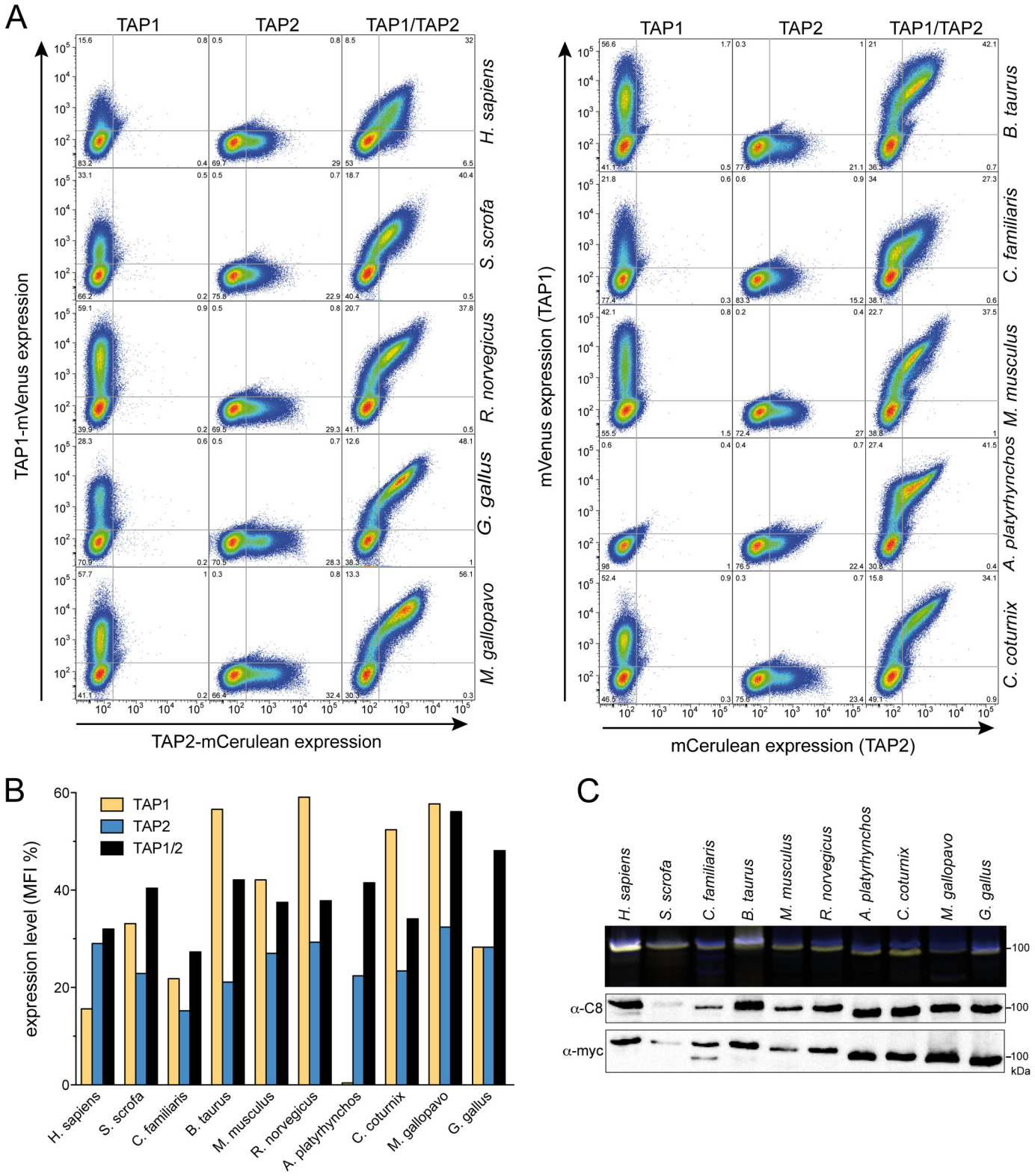

FIGURE 2. **Avian and mammalian TAP1 and TAP2 are expressed in human cells.** HEK293T cells were cotransfected with TAP1 and TAP2 from different species. *A*, expression levels of TAP1 and TAP2 were monitored by flow cytometry. The dot blots represent transfections with TAP1 (*left*), TAP2 (*middle*) and TAP1/TAP2 (*right*) from different species. The fluorescence gates of mVenus and mCerulean are shown as *gray lines*, and numbers represent gate populations in %. *A. platyrhynchos* TAP1 lacking its correct 3′-region did not express. To analyze the expression and function of TAP2 from *A. platyrhynchos*, it was coexpressed with *G. gallus* TAP1. *B*, expression levels of TAP1 (*blue*), TAP2 (*yellow-orange*), as well as TAP1/TAP2(*black*) are summarized. *C*, expression of TAP1-mVenus and TAP2-mCerulean was analyzed by SDS-PAGE in-gel fluorescence (TAP1: *yellow* and TAP2: *blue*) and immunoblotting. *White signals* indicate a spectral overlap of the YFP and the CFP channel. TAP1 and TAP2 were detected with a C8- and myc-antibody, respectively.

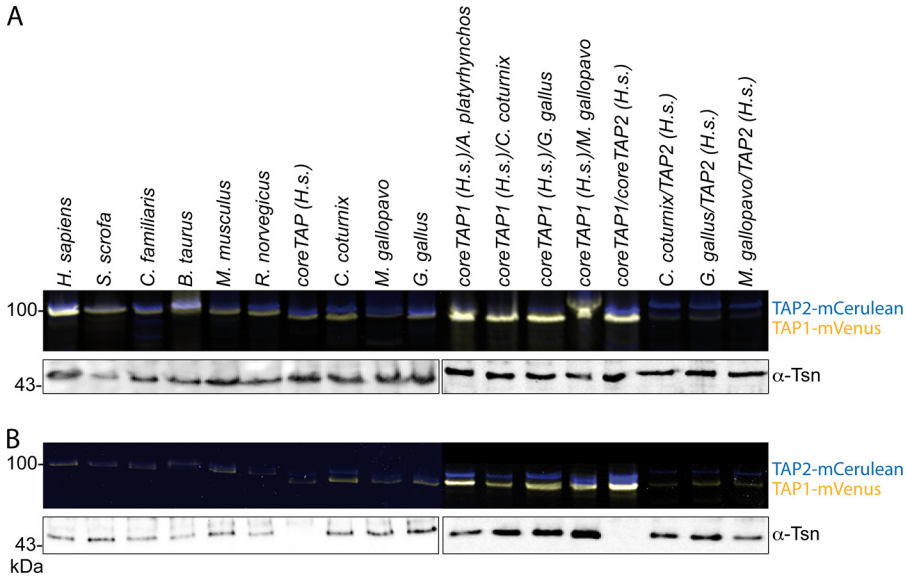

FIGURE 3. **Avian and mammalian TAP complexes interact with tapasin.** Immunodetection of human tapasin after tandem-affinity purification of avian and mammalian TAP complexes expressed in HEK293T. Expression of TAP1 is colored in *yellow*, that of TAP2 in *blue*. A *white signal* indicates a spectral overlap of the YFP and the CFP channel. Tapasin was detected by the antibody 7F6. The input after solubilization (*A*) and the tandem-affinity purified PLC (*B*) are shown.

As a proof of principle, we also elucidated whether chimeric avian/human TAP complexes can assemble heterodimers and interact with tapasin. We transfected HEK293T cells with human tapasin and combinations of avian TAP1 and human TAP2, or human coreTAP1 together with avian TAP2 (Fig. 3, *right panel*). Based on this strategy, tapasin can only bind to the TMD0 of an avian TAP subunit. In all cases, tandem-affinity purified TAP1/2 complexes bound tapasin. These results lead to the following conclusions: First, the dimerization interface between avian and human TAP1 and TAP2 is complementary over a long distance in evolution. Secondly, all TAP complexes are capable to assemble the peptide-loading complex via specific recruitment of tapasin, indicating that the modules required for assembly of the peptide-loading complex are conserved in evolution across different classes of jawed vertebrates.

Mammalian TAP Subunits Are Functional in Antigen Processing—To investigate whether the ability to assemble TAP complexes, even across different species, correlates with TAP function, we transfected human skin fibroblast cell lines that are deficient either in TAP1 (BRE169 (22)) or in TAP2 (STF169 (23)) with individual mammalian TAP genes. A deficiency in either TAP gene leads to a down-regulation of MHC I molecules at the cell surface, termed type I bare lymphocyte syndrome (BLS). We have previously shown that transfection of these cells with the lacking human TAP subunit leads to complementation of functional TAP complexes and increase of peptide-loaded MHC I molecules at the plasma membrane (24).

Transfection of TAP1-negative BRE169 cells with TAP1 genes of various mammalian species led to an up-regulation of MHC I complexes at the cell surface by more than one order of magnitude (Fig. 4*A*). Only pig TAP1 reconstituted a slightly lower MHC I surface expression. In contrast, the transfection of TAP1-deficient cells with any of the mammalian TAP2 genes did not influence the MHC I surface expression (data not shown). Thus, any combination of mammalian TAP1 with endogenous human TAP2 led to the formation of a functional TAP complex.

In line with these observations, the transfection of TAP2-deficient STF169 cells with mammalian TAP2 genes led to an up-regulation of MHC I surface expression (Fig. 4*B*), whereas the transfection with any of the mammalian TAP1 did no restore the surface expression of MHC I (data not shown). Thus, mammalian TAP2 form a functional complex with endogenous human TAP1, restoring antigen processing in TAP2-deficient cells. As previously observed, the effect on MHC I surface expression is less pronounced in TAP2-deficient cells as compared with TAP1-deficient cells (24). This is likely caused by differences in the expression level of endogenous components of the peptide-loading complex, *e.g.* TAP, tapasin, or MHC I in BRE169 and STF169 cells.

Cotransfection of TAP1/2 led to a similar up-regulation of MHC I surface expression as compared with the single transfection of the corresponding missing gene in the human TAP-deficient cells (Fig. 4, *C* and *D*), demonstrating that these cells are a *bona fide* expression host for TAP subunits of the different species used. The restoration of antigen processing in TAP-negative cells by the corresponding TAP subunit is summarized in Fig. 4*E*. In conclusion, the interface of TAP1 and TAP2 across different mammalian species is complementary promoting assembly of functional peptide translocation and loading complexes.

Avian TAP Complexes Are Functional but Not across Taxa—Although all avian TAP subunits were well expressed in human cells, transfection of avian TAP1 into TAP1-deficient human cells did not restore MHC I surface expression (Fig. 5*A*). Similarly, none of the avian TAP2 genes did complement MHC I loading in TAP2-deficient human cells (Fig. 5*B*). Although avian TAP subunits formed complexes with their human counterparts (see Fig. 3), these complexes did not shuttle antigenic peptides to MHC I molecules for surface presentation. Expression of TAP2 and TAP1 in TAP1- and TAP2-deficient cells,

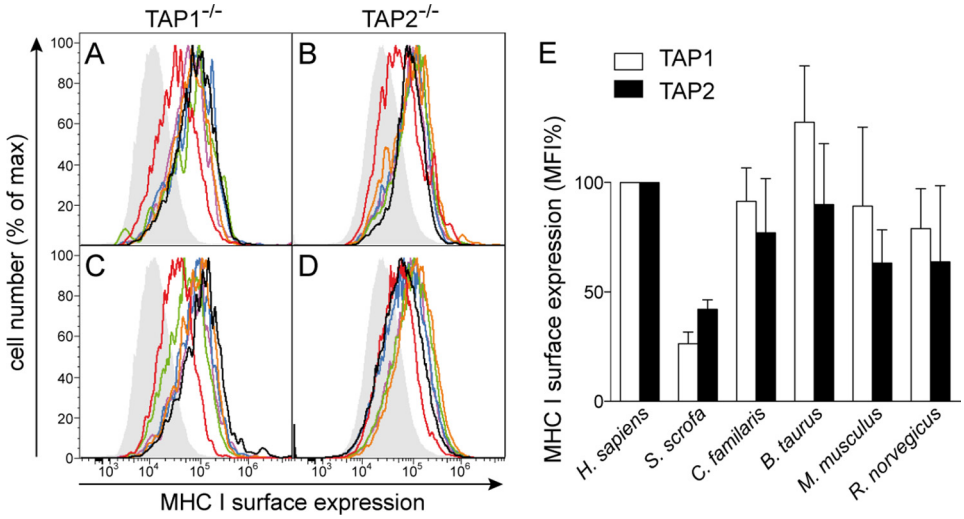

FIGURE 4. **All mammalian TAP1 and TAP2 restore MHC I surface expression in TAP-deficient cells.** TAP1-deficient BRE169 (*A*) or TAP2-deficient STF169 cells (*B*) were transfected with mammalian TAP1 or TAP2, respectively. Cells were gated on the expression of TAP1-mVenus (BRE169) or TAP2-mCerulean (STF169), respectively, and the MHC I surface expression is shown. MHC I were stained with a mouse antibody W6/32 and a PerCP-Cy5.5-coupled anti-mouse secondary antibody. *H. sapiens* (*black*), *S. scrofa* (*red*), *C. familiaris* (*orange*), *B. taurus* (*green*), *M. musculus* (*blue*), and *R. norvegicus* (*pink*), mock transfections are shown in *gray*. After cotransfection of BRE169 (*C*) or STF169 cells (*D*) with TAP1/TAP2, double positive cells were gated by the fluorescence of TAP1-mVenus and TAP2-mCerulean, and MHC I surface expression is shown. *E*, the restoration of MHC I surface expression in TAP1- and TAP2-deficient cells by the corresponding TAP1 (*white bars*) and TAP2 subunits (*black bars*). Mean fluorescence intensities (MFI%) and the S.D. are provided from two independent experiments.

respectively, did not lead either to an up-regulation of MHC I surface expression, excluding the possibility that functional TAP1-TAP1 or TAP2-TAP2 dimers are formed (data not shown).

In contrast to single transfections, coexpression of avian TAP1 and TAP2 genes in either TAP1-deficient (Fig. 5*C*) or TAP2-deficient cells (Fig. 5*D*) restored MHC I surface expression. This up-regulation was almost identical when compared with the complementation by human TAP1/TAP2. Since the peptide specificity of chicken TAP is restrictive and overlaps with the MHC I specificity of the same haplotype (5), restoration of the MHC I surface expression indicates that the chicken translocation machinery provides a subset of peptides compatible for MHC I loading in human cells. Of note, we analyzed chicken TAP1 and TAP2 of the B4 and B21 haplotype, respectively, of which the peptide specificity has not been analyzed (5). In addition, TAP from other poultry restored the MHC I surface expression in TAP-deficient cells, demonstrating that the peptide pool in human cells is generally compatible with the specificity of avian TAP and that human MHC I molecules are efficiently loaded by the subset of peptides translocated by avian TAP.

Notably, all combinations of avian TAP1 and TAP2 led to an identical increase of peptide-loaded MHC I surface expression of TAP-deficient cells (Fig. 5, *E–G*). For example, cotransfections of TAP1-deficient cells with *e.g.* TAP1 from *C. coturnix* and TAP2 from *A. platyrhynchos*, *G. gallus*, or *M. gallopavo*, respectively, had the same positive effect on MHC I surface expression as the cotransfection with TAP2 from *C. coturnix*. These results prove that all avian TAP1 and TAP2 form functional TAP complexes and assemble a PLC for efficient peptide loading of MHC I molecules.

We finally analyzed peptide translocation into the ER-lumen in semi-permeabilized TAP1-negative BRE169 cells (Fig. 5*H*). Mammalian TAP1 restored ATP-dependent peptide translocation into the ER. In contrast, chicken TAP1 was only functional in combination with avian TAP2 and not with endogenous or overexpressed human TAP2.

DISCUSSION

In this study, we delineated the basic function of representative mammalian and avian TAP complexes. We demonstrated that TAP1 and TAP2 form heterodimers across taxa. We established that all mammalian and avian TAP subunits harboring an extra N-terminal TMD0 are able to bind tapasin and thus recruit MHC I molecules into the peptide-loading complex. Despite this well conserved function, antigen translocation by the TAP complex is restricted to one taxon. The functional comparison of mammalian and avian PLCs provides a first insight into the evolution of the adaptive immune system.

In birds, the evolution of the MHC locus has converged in one predominantly expressed MHC I molecule with polymorphic TAP and tapasin. This resulted in a haplotype specific peptide translocation and presentation with strong resistance toward certain pathogens (4, 5, 25). In contrast, evolution of TAP, tapasin, and MHC I has gone a different route in mammals. The immune response is dominated by the high allelic polymorphism of MHC I. TAP has evolved with low allelic polymorphism (26–30), while offering a broad peptide specificity. This leads to an expansive epitope repertoire presented at the cell surface by up to six different MHC alleles, but a stronger association with autoimmunity (4).

The assembly of transport incompetent avian/human TAP1/2 heterodimers suggests that both TAP subunits provide the specific interaction sites, but the chimeric ABC transporter cannot run through the peptide translocation cycle. A sequence alignment illustrates differences especially in the coupling helixes (CH) between representative TAP subunits of both classes, whereas the key motifs in ATP binding and hydrolysis are conserved, displaying a degenerate ATP-bind-

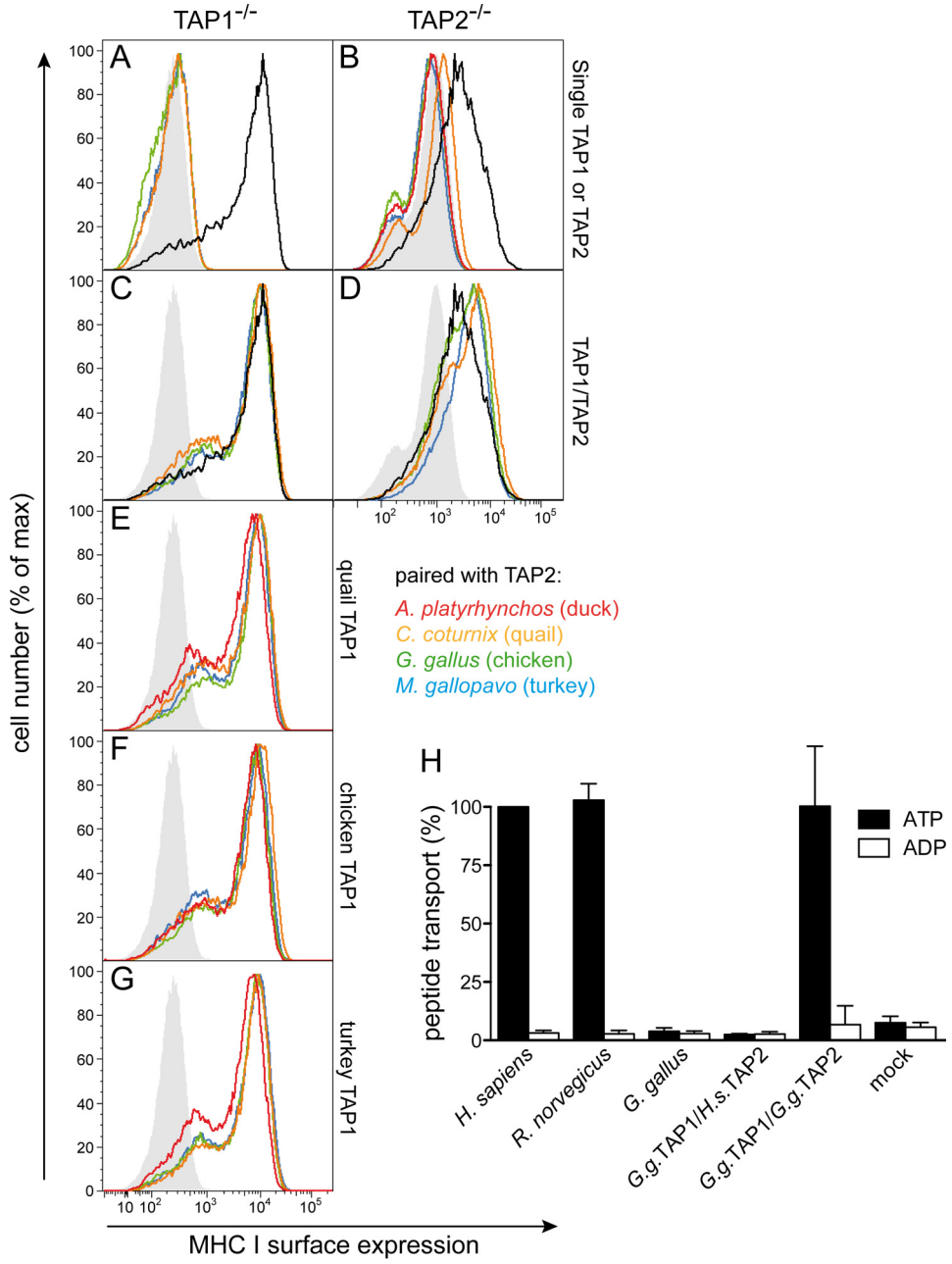

FIGURE 5. **Avian TAP1/TAP2 restore MHC I peptide loading and surface expression.** After transfections of TAP1-deficient BRE169 (*A*) or TAP2-deficient STF169 cells (*B*) with avian TAP1 or TAP2, respectively, the cells were gated on the expression of TAP1-mVenus (BRE169) or TAP1-mVenus (STF169). MHC I was stained with a mouse antibody W6/32 and an APC-coupled anti-mouse secondary antibody. Colors are: *H. sapiens* (*black*), *A. platyrhynchos* (*red*), *C. coturnix* (*orange*), *G. gallus* (*green*), and *M. gallopavo* (*blue*). TAP1-deficient BRE169 (*C*) or TAP2-deficient STF169 cells (*D*) were cotransfected with TAP1/TAP2 of the same species. TAP1-deficient BRE169 were cotransfected with TAP1 from *C. coturnix* (*E*), *G. gallus* (*F*), or *M. gallopavo* (*G*) in combination with TAP2 from *A. platyrhynchos* (*red*), *C. coturnix* (*orange*), *G. gallus* (*green*), and *M. gallopavo* (*blue*), respectively. Double positive cells were gated by the fluorescence of TAP1-mVenus and TAP2-mCerulean, and the MHC I surface expression is shown. *H*, ATP-dependent peptide transport in TAP1-deficient BRE169 cells transfected with TAP1-mCherry or combinations of TAP1-mCherry and TAP2-mCerulean. Cells transfected with mCherry (*mock*) are used as control. Transport is normalized to human TAP1 and the S.D. is provided from three independent experiments.

ing site I and a catalytic ATP-binding site II in all TAP sequences. CH1 is remarkably different between avian and mammalian TAP1, whereas CH2 of TAP2 shows less variation. Since the coupling helices are supposed to mediate the conformational coupling between the TMDs and the NBDs (13, 14), the sequence variations in CH1 and CH2 of avian TAP1 and TAP2, respectively, might inhibit this allosteric crosstalk in chimeric avian/human transporters. Thus, although their interface is compatible for TAP1/TAP2 assembly, conformational changes that are essential for peptide translocation are hindered. For structural studies, this makes chimeric TAP a very interesting target since the inhibition of a translocation cycle may arrest TAP in a certain conformation like viral inhibitors, *e.g.* BNLF2a and US6, do (31–33). Therefore, these arrested chimeric complexes are presently examined for x-ray analysis of the TAP complex, which has been unsuccessful over the past years. Remarkably, although the TMD0 of avian and mammalian TAP sub-

units do not share significant sequence similarities, the interaction between TAP and tapasin is conserved across mammals and birds. Notably, all avian TAP1 lack the tapasin interaction platform TMD0. However, reptiles, amphibians, bony fish, and cartilaginous fish do contain TMD0 at both TAP subunits.

Tapasin is crucial for peptide editing as it keeps MHC I molecules in a peptide-receptive conformation (25, 34). Notably, the interaction between tapasin and ERp57 within avian peptide-loading complexes is different from the mammalian PLC, since Cys-95 of mammalian tapasin, forming an intermolecular disulfide bond with Cys-57 of ERp57, is replaced by a serine residue in all avian tapasin. However, despite important substitutions in tapasin, which are directly involved in the assembly of other components of the PLC, *e.g.* Cys-95, and the low sequence conservation of the TMD0 from different classes, this study demonstrates for the first time the importance and conservation of the interaction between TAP and tapasin for PLC assembly and translocation function of the TAP complex in mammalian and avian species.

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