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Association of ERp57 with Mouse MHC Class I Molecules Is Tapasin Dependent and Mimics That of Calreticulin and not Calnexin

Michael R. Harris,* Lonnie Lybarger, † Yik Y. L. Yu, † Nancy B. Myers, † and Ted H. Hansen 2†

Before peptide binding in the endoplasmic reticulum, the class I heavy (H) chain-β2-microglobulin complexes are detected in association with TAP and two chaperones, TPN and CRT. Recent studies have shown that the thiol-dependent reductase, ERp57, is also present in this peptide-loading complex. However, it remains controversial whether the association of ERp57 with MHC class I molecules precedes their combined association with the peptide-loading complex or whether ERp57 only associates with class I molecules in the presence of TPN. Resolution of this controversy could help determine the role of ERp57 in class I folding and/or assembly. To define the mouse class I H chain structures involved in interaction with ERp57, we tested chaperone association of Ld mutations at residues 134 and 227/229 (previously implicated in TAP association), residues 86/88 (which ablate an N-linked glycan), and residue 101 (which disrupts a disulfide bond). The association of ERp57 with each of these mutant H chains showed a complete concordance with CRT, TAP, and TPN but not with calnexin. Furthermore, ERp57 failed to associate with H chain in TPN-deficient 220 cells. These combined data demonstrate that, during the assembly of the peptide-loading complex, the association of ERp57 with mouse class I is TPN dependent and parallels that of CRT and not calnexin. The Journal of Immunology, 2001, 166: 6686–6692.

The biosynthesis of oligomeric proteins entering the secretory pathway is facilitated by chaperone proteins in the endoplasmic reticulum (ER)3 that are thought to provide quality control to insure correct folding. For example, MHC class I molecules require assembly in the ER of three subunits, a heavy (H) chain, β2-microglobulin (β2m), and peptide ligand (1). Recent studies demonstrate that assembly intermediates of class I are associated with several ER proteins including calnexin (CXN), calreticulin (CRT), TAP, tapasin (TPN), and ERp57 (2). The dynamics of ER chaperone interactions with class I assembly intermediates and the selective roles of each ER chaperone in class I biogenesis remain topics of considerable interest and controversy.

Although several details remain unclear or controversial, the temporal order of events involved in class I assembly is starting to emerge. As the MHC class I H chain is translocated into the ER, intrachain disulfide bonds are formed within the α2 and α3 domains (3). Interestingly, the formation of these two H chain disulfide bonds may be temporally distinct (4, 5). This observation raises the possibility that the α3 and α2 disulfide bond formation may correspond with H chain interaction with β2m and peptide, respectively, according to a kinetic model (6, 7). Both non- and disulfide-bonded H chains can be detected in association with CXN, an ER chaperone implicated in either stabilizing free H chains and/or promoting H chain assembly with β2m (3, 8, 9). After β2m assembly, class I-β2m heterodimers are detected in association with CRT-TAP-TPN, a complex referred to here as the peptide-loading complex (2, 10–16). The order of assembly of this peptide-loading complex is unclear (2). However, once a peptide binds to form the fully assembled class I molecule, it is no longer associated with the peptide-loading complex, thus releasing class I molecules from the ER for transit to the cell surface (10–12).

Several H chain mutations have been described that disrupt its interaction with members of the peptide-loading complex. For example, α1 residue N86 (17), α2 residues 116 (18) and 128–136 (19–21), and α3 residues 223–229 (12, 22, 23) have all been implicated in H chain interaction with the peptide-loading complex. However, it remains unclear which, if any, of these are direct sites of interaction with TAP, TPN, or CRT. Part of the problem in defining direct interaction sites is the fact that cooperative binding among members of the peptide-loading complex may be required for their stable association with class I (21, 24). Furthermore, these mutagenesis approaches have been technically limited because of the lack of reagents capable of precipitating ER proteins while associated with class I molecules and the lack of reagents capable of identifying specific class I alleles while associated with ER molecules. Despite these limitations, a model has been proposed whereby CRT specifically interacts with the N-linked glycan at H chain residue 86, whereas TPN interacts with residue 128–136 and 223–229 in the α2 and α3 domains, respectively (25). However, this model must be considered speculative because of the aforementioned cooperative binding issue as well as the fact that the model does not consider the contribution of the most recently identified participant in class I assembly, ERp57 (26–28).

ERp57 has thiol-dependent reductase activity (29), cysteine-dependent protease activity (30, 31), and is known to interact with glycoproteins in a manner that can involve forming complexes

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*Abbreviations used in this paper: ER, endoplasmic reticulum; β2m, β2-microglobulin; CXN, calnexin; CRT, calreticulin; TPN, tapasin.
with either CXN and CRT (32–34). Three recent reports demonstrated that ERp57 is detected in association with class I molecules before peptide binding (26–28). However, important details regarding this association remain controversial, such as whether ERp57 association with class I is dependent upon TNP or whether ERp57 can associate with class I before its association with other members of the peptide-loading complex. Resolution of this controversy could help define the primary function of ERp57, which, reportedly, may include H chain disulfide bond formation, peptide trimming, or ER degradation of misfolded class I proteins (26).

In this report, we study the association of a series of H chain mutations with ERp57, TPN, and other ER molecules associated with class I during its assembly. This analysis includes mutations in all three extracellular domains that have been reported to disrupt H chain association with CRT-TNP-TAP and have not been previously tested for association with ERp57. The advantage of a mutagenesis approach is that a particular mutant may reduce or disrupt class I interaction with a particular ER protein and may thus prolong associations with other ER proteins that may otherwise be difficult to detect. In this regard, perhaps the most interesting mutation tested is C101S, because it prevents the disulfide bridge in the α2 domain that is a critical for the formation of the peptide binding groove. The simplest model consistent with these findings suggests that, during the assembly of the peptide-loading complex, ERp57 associates with CRT, and ERp57-CRT complexes only bind class I-β, m in the presence of TPN.

Materials and Methods

Cell lines

All the cell lines used in this report have been previously described. Mutant forms of Ld expressed in various cell lines are referred to by a single letter designation of native amino acid followed by the position number, which is then followed by a single letter designation of the newly introduced amino acid. The mouse L cell transfectants used in this study were: L-Ld (34), L-Ld D227K and L-Ld D227K/E229K (12), L-Ld K131D and L-Ld T134K (21), L-Ld N86Q, L-Ld N176Q, L-Ld S88W (17), and L-Ld C101S (16). Two human lymphoblastoid cell lines transfected with the Ld cDNA were also used, LCL721.220-Ld and LCL721.221-Ld.

Antibodies

The Abs used in this study are listed in Table I. Briefly, mAb 30-5-7 (37) was used to precipitate fully assembled forms of Ld, and mAb 64-3-7 (38) was used to precipitate open forms of Ld indiscriminate of their β, m association. The mAb 64-3-7 was also used in Western blot analyses, in which it detects total Ld independent of conformation (12). The mAb 148.3 (42) to human TAP was a kind gift of Dr. R. Tampe, and mAb 1478 (17) was produced to a peptide REATNPPIIQEEKPKKKKKAQEDL representing the C-terminal amino acids of mouse ERp57 (28). The specificity of this reagent was determined based on the appropriate m.w. of the precipitin band and the fact that it was detected in association with incompletely and not fully assembled class I molecules. This anti-mouse ERp57 (number 2890) was used for precipitation or Western blotting. A rabbit polyclonal Ab (number 2668) and an Armenian hamster mAb (SD3) were produced to peptide EDAGGGGLSKKPATLLRHHK被人ESERp57 (number 2668) was used for precipitation and Western blot assays, whereas the mAb SD3 to mouse TNP was used for Western blotting.

Immunoprecipitation

Cells were washed three times in cold PBS and were lysed in Tris-buffered saline that contained 1% digitonin (Wako, Richmond, VA) with 20 mM iodoacetamide (Sigma, St. Louis, MO) and 0.5 mM freshly added PMSF. The lysates were supplemented with a saturating amount of rab-

Table I. Abs used in this study

<table>
<thead>
<tr>
<th>Specificity</th>
<th>Designation</th>
<th>Source (Ref.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-assembled Ld mAb</td>
<td>30-5-7</td>
<td>(35, 38)</td>
</tr>
<tr>
<td>Anti-open forms Ld mAb</td>
<td>64-3-7</td>
<td>(35, 39–41)</td>
</tr>
<tr>
<td>Ra anti-CXN polyclonal</td>
<td>SPA-860</td>
<td>StressGen Biotechnologies, Victoria, Canada</td>
</tr>
<tr>
<td>Ch anti-CRT polyclonal</td>
<td>PA1-903</td>
<td>Affinity BioReagents, Golden, CO</td>
</tr>
<tr>
<td>Ra anti-ERp57 polyclonal</td>
<td>2890</td>
<td>C-terminal 24 residues of ERp57</td>
</tr>
<tr>
<td>Ra anti-hu TAP polyclonal</td>
<td>1478</td>
<td>C-terminal 14 residues of hu TAP-1 (17)</td>
</tr>
<tr>
<td>Anti-hu TAP mAb</td>
<td>148.3</td>
<td>Gift of R. Tampe (42)</td>
</tr>
<tr>
<td>Ra anti-m TAP</td>
<td>502</td>
<td>Residues 11–34 m TNP</td>
</tr>
<tr>
<td>Anti-m TNP polyclonal</td>
<td>2668</td>
<td>Residues 11–34 m TNP</td>
</tr>
<tr>
<td>Ha anti-m TNP mAb</td>
<td>5D3</td>
<td>American Type Culture Collection, Manassas, VA (43)</td>
</tr>
<tr>
<td>Anti-β, m mAb</td>
<td>BBM-1</td>
<td></td>
</tr>
</tbody>
</table>

a Ra, Rabbit; Ch, chicken; Ha, Armenian hamster; m, mouse; hu, human.

b New reagent.
the mutagenesis approach reported here, new reagents were developed including a rabbit serum to mouse ERp57 and mouse TPN that can precipitate these ER proteins while associated with class I (14). Furthermore, a new hamster mAb that specifically detects mouse TPN by Western blotting was developed (Fig. 1). With these combined reagents, the association of mutant H chains with salient ER proteins could be rigorously tested.

Mutations in H chain residues 227 and 229 disrupt association with ERp57, TPN, CRT, but not CXN

Several studies have now demonstrated, using mouse Ld (12), Db (22), or Dd (23) molecules, that mutations in the α3 domain can disrupt association with TPN-TAP. To determine whether α3 mutation also affects ERp57 association, lysates of cells expressing Ld H chains with a single mutation at residue D227K or a double mutation at residues D227K and E229K were tested. Results obtained by precipitating Ld, Ld D227K, or Ld D227K/E229K and then blotting with anti-CXN, anti-CRT, anti-ERp57, anti-TAP, or anti-TPN show that these mutant H chains display strong association with ERp57, CRT, TAP and TPN but not with CXN. Lysates of L-Ld, L-Ld D227K, and L-Ld D227K/E229K cells were precipitated with mAb 64-3-7 and blotted with anti-CXN, anti-CRT, anti-ERp57, anti-TAP, and anti-TPN. As shown in Fig. 2, Ld, Ld D227K, and Ld D227K/E229K H chains were detected in all three lysates, and all three of these H chains displayed strong association with ERp57. By contrast with these findings, the α3 mutant H chains displayed weaker (Ld D227K) or no (Ld D227K/E229K) association with ERp57 (Fig. 2) or with CRT, TAP, or TPN, as also previously reported (12, 21). To extend these findings, precipitations were also performed using anti-ERp57 or anti-TPN followed by blotting for Ld H chain association. In data not shown, significantly less α3 mutant Ld H chain was detected in association with either TPN or ERp57 compared with wild-type Ld H chains. It is noteworthy that, in all of our comparisons, the double mutation was considerably more efficacious at ablating associations with ER proteins relative to the single mutation. This finding is consistent with an earlier report by Kulig et al. (22). In any case, ERp57 association with these α3 mutants clearly parallels that of TAP-TPN-CRT. Furthermore, based on studies of α3 mutations of Dd that were associated with CRT and not TPN-TAP, it was predicted that the α3 domain may be a site on the class I H chain that directly interacts with TPN. In such a model, it is assumed that only TPN, and not TAP, directly contacts class I (14).

Residue 134 was originally shown to affect class I association with the peptide-loading complex in studies of HLA-A2.T134K (19, 20). More recent studies in our laboratory have defined the extent of the region surrounding residue 134 involved in TAP-TPN-CRT association by mutagenesis of Ld (21). In this previous report, amino acid substitutions of Ld residues 128–136 significantly reduced association with TAP-TPN-CRT with the exception of Ld K131D. Interestingly, the K131D mutation appeared as a hyper-morph in terms of loading complex association, because it had enhanced association with TAP-TPN in comparison with wild-type Ld (21). Furthermore, of the mutations that reduced association, T134K was among the most efficacious. To determine whether the K131D and T134K mutations affected ERp57 association, lysates of L cells expressing wild-type Ld, Ld K131D, or Ld T134K were precipitated with mAb 64-3-7 and blotted with anti-CXN, anti-CRT, anti-ERp57, anti-TAP, and anti-TPN. As shown in Fig. 3A, Ld and Ld K131D molecules were found to be associated with all ER proteins tested. By contrast, Ld T134K showed no detectable association with ERp57, TAP, TPN, and CRT. Importantly, each of these cell lines synthesized readily detectable Ld H chains and displayed high levels of CXN association (Fig. 2A). However, the level of Ld H chains expressed by each of these cell lines was clearly disparate, potentially influencing the detection of associated ER proteins. Thus, this experiment was repeated using samples in which the Ld H chains were normalized based on expression differences determined by densitometry (see Fig. 3B legend for details). Normalizing for Ld H chain expression resulted in similar qualitative differences in ER protein associations as obtained using cell equivalents (compare Fig. 3, A and B). Furthermore, normalizing for Ld H chain provided clear evidence that Ld K131D had increased association with CRT-ERp57-TAP-TPN, whereas Ld T134K lacked association with these same ER proteins (Fig. 3B). Interestingly, CXN associated comparably with Ld, Ld K131D, or Ld T134K. Thus, ERp57 association with the K131D and T134K mutants correlates precisely with that of the peptide-loading complex (TAP-TPN-CRT) and not CXN. Based on studies

**FIGURE 1.** Specificity of the rabbit polyclonal Ab (number 2668) and hamster mAb 5D3 for mouse TPN. Cell lysates from 221, 220, and 220 transfected with mouse TPN were tested by Western blotting with the indicated Ab. These Abs were raised against a region of mouse TPN that shows a divergence from human TPN. Furthermore, this region is absent in the truncated human TPN produced by .220 cells (51). Thus, the finding that no unique bands are present in .220 cells (human TPN-positive) relative to .220 cells demonstrates that these Abs are mouse TPN specific and that the common bands seen with rabbit antiserum are nonspecific.

**FIGURE 2.** Mutation at residues in the α3 domain disrupt association with ERp57, CRT, TAP and, TPN but not with CXN. Lysates of Ld, Ld D227K, and Ld D227K/E229K cells were precipitated with mAb 64-3-7 to specifically detect open forms of Ld. Aliquots representing cell equivalents of each of these precipitates were then blotted with the Ab listed along the left side. Specific blotting reagents used for this experiment were anti-CXN (SPA-860), anti-CRT (PA1-903), anti-ERp57 (2890), anti-TAP (502), anti-TPN (mAb 5D3), and anti-Ld (64-3-7).
The N-linked glycan at residue 86 is specifically required for H chain association with ERp57 and CRT but not CXN

We previously showed that association of L\(^d\) with the complex of TAP-TPN-CRT requires the N-linked oligosaccharide at residue 86 (17). This conclusion was based on mutagenesis studies of L\(^d\), a class I molecule with three N-linked glycans, one in each of the three external domains. In this previous study, L\(^d\) mutation at N86 was reported to ablate association with TPN-TAP-CRT. To determine whether ERp57 association with L\(^d\) shows the same glycan dependency, two mutations that ablate the N86-linked glycan were tested, N86K and S88W. Thus, lysates of cells expressing wild-type L\(^d\) or L\(^d\) N86K and L\(^d\) S88W mutant molecules were precipitated with Ab to the various ER proteins and blotted with mAb 64-3-7 to detect associated L\(^d\) H chains (Fig. 4C). Using each of these approaches, neither the L\(^d\) N86K or L\(^d\) S88W mutants were found to be associated with ERp57, CRT, TPN, or TAP. It should also be noted that both mutants were associated with CXN. Thus, these combined studies clearly show that ERp57, like other members of the loading complex, is dependent upon the N86 glycan for association with the class I H chain.

Based on the lectin activity of CRT (33), it was proposed that N86 is specifically required for CRT to interact with the class I H chain (17). Given the validity of this conclusion, the findings in Fig. 3 indicated that TPN-TAP and ERp57 are dependent upon CRT to form stable, steady-state associations with L\(^d\). Interestingly, CXN binds H chains lacking the N86 glycan (Fig. 3A and Ref. 17). However, CXN association with L\(^d\) is clearly increased by N-linked glycosylation (M. R. Harris and T. H. Hansen, unpublished observation) as predicted from studies using glucosidase inhibitor castanospermine in studies of other class I molecules (45, 46). The advantage of the mutagenesis approach taken here is that L\(^d\) has three N-linked glycans, allowing us to clearly demonstrate that the N86 glycan is uniquely required for H chain association with CRT and the loading complex. By contrast, preventing complete glycosylation can result in aggregation and nonspecific association of aglycosylated proteins with CXN (47). The fact the CRT requires the glycan in the \(\alpha\)1 domain of the H chain may be influenced by the fact that CRT is a soluble protein, whereas CXN is membrane anchored. Furthermore, the location of the H chain \(\alpha\)1 domain glycan positions CRT near the site of peptide loading, possibly rendering CRT better able to interact with other members of the peptide-loading complex.

**FIGURE 3.** Mutation in the \(\alpha\)2 domain at residue 134 disrupts H chain association with ERp57 and CRT but not CXN

A, Aliquots of lysates representing cell equivalents of L-L\(^d\), L-L\(^d\) K131D, and L-L\(^d\) T134K were precipitated with 64-3-7 and blotted with the various Abs to ER proteins (specific Abs listed in Fig. 1 legend). B, Same as A except quantity of sample loaded was normalized according to the relative amount of L\(^d\) H chain that was determined by densitometry. Relative levels of L\(^d\) H chain are determined to be 8:1:1 for L-L\(^d\), L-L\(^d\) K131D, and L-L\(^d\) T134K, respectively.

**FIGURE 4.** The N-linked glycan at residue 86 is required for H chain association with ERp57, CRT, TPN, and TAP but not with CXN. A, Aliquots of lysates representing cell equivalents of L-L\(^d\), L-L\(^d\) N86K, and L-L\(^d\) S88W were precipitated with mAb 64-3-7, and then each precipitate was blotted with the Ab listed along the left (see Fig. 1 legend for specific Ab used for blotting). B, Same as A except quantity of sample loaded was normalized according to the relative amount of L\(^d\) H chain that was determined by densitometry to be 16:2:1 for L-L\(^d\), L-L\(^d\) N86K, and L-L\(^d\) S88W, respectively. C, Normalizing for the amount of L\(^d\) H chains, aliquots of lysates of L-L\(^d\), L-L\(^d\) N86K, and L-L\(^d\) S88W were precipitated with anti-TAP (502), anti-TPN (2668), anti-ERp57 (2890), or anti-open L\(^d\) (64-3-7). Each precipitate was then blotted with mAb 64-3-7.
64-3-7 and then blotted with Abs to various ER proteins. As shown in Fig. 5A, L^d C101S was found to be highly associated with CXN. By contrast, ERp57, CRT, TPN, and TAP displayed substantially reduced association with L^d C101S relative to wild-type L^d. Furthermore, when these respective lysates were precipitated with anti-TAP, anti-TPN, or anti-ERp57 (Fig. 5B), only wild-type L^d, and not L^d C101S, molecules were detected by blotting for L^H H chains. Thus, C101S mutation of L^d severely reduces its ability to associate with ERp57 as well as TAP, TPN, and CRT but not CXN. It should be noted that failure of L^d C101S to associate with TAP-TPN-CRT proteins confirms an earlier report (16) and is consistent with studies that used DTT to identify H chain disulfide bond intermediates (5, 48, 49). These combined studies indicate that the α2 disulfide bond, like the α3 disulfide bond, likely formed before steady-state association of H chain with TAP-TPN-CRT. The original observation reported here is that formation of the H chain α2 disulfide bond is also required for ERp57 to display steady-state association. The fact that the C101S mutation does not increase H chain association with ERp57 raises questions regarding the role of ERp57 in formation of the disulfide bond in the α2 domain of the class I H chain.

**FIGURE 5.** Mutation C101S disrupts H chain association with ERp57, CRT, TPN, and TAP but not with CXN. A, Samples representing cell equivalents of lysates from L-L^d C101S and L-L^d were precipitated with mAb 64-3-7, and aliquots of each of these precipitates were blotted with the reagent listed along the left (specific identity of reagents used listed in legend of Fig. 1). B, Aliquots of lysates of L-L^d C101S and L-L^d were immunoprecipitated with anti-ERp57 (2890), anti-TAP (502), anti-TPN (2668), or anti-L^d (mAb 64-3-7). All precipitates were then blotted with mAb 64-3-7.

**ERp57 association with L^d is not detected in human TPN-deficient .220 cells**

There are conflicting reports in the literature as to whether human class I H chains are detected in association with ERp57 in TPN-deficient .220 cells. Furthermore, to the best of our knowledge, there are no reports of the TPN dependency of ERp57 association with mouse class I. To address this question, ERp57-L^d association was compared in TPN-deficient .220-L^d and TPN-positive .221-L^d cells. From lysates of each of these cell lines, L^d molecules were precipitated with either mAb 30-5-7, which detects fully assembled L^d-β_m-peptide heterotrimers, or mAb 64-3-7, which detects open forms of L^d H chains in a β_m-independent manner. In this experiment, .220-L^d and .221-L^d lysates were also precipitated by a mAb to human TAP (148.3) and a mAb to human β_m (BBM1). As indicated in Fig. 6, each of these precipitates was blotted with anti-human TAP or anti-human ERp57. The experiment shown in Fig. 6 makes several important points. As previously noted, only 64-3-7-positive forms of L^d are detected in association with TAP (12), and this association is only seen in .221-L^d cells and not .220-L^d cells (36). Interestingly, ERp57-L^d association showed the identical result. Thus, L^d association with ERp57, like TAP, is TPN dependent. Furthermore, β_m association with ERp57 was only observed in .221-L^d, suggesting that L^d-β_m heterodimers require TPN to maintain stable association with ERp57. Furthermore, these data show that ERp57 only interacts with L^d-β_m heterodimers and not free H chains.

In summary, multiple lines of evidence are presented showing that ERp57 association with mouse L^d correlates precisely with TAP-TPN-CRT and not CXN. This finding clearly validates the conclusion that ERp57 is a bona fide member of the peptide-loading complex (26–28) and, furthermore, suggests a model consistent with the data reported here as well as most of the published literature (2). In this model, ERp57-H chain-β_m complexes are only associated with CRT and not CXN. Given our findings as well as certain other published reports using .220 cells (26), we propose that ERp57-CRT complexes are TPN dependent in their association with class I-β_m complexes. This latter dependency is consistent with published findings that CRT is also TPN dependent in its association with class I (14, 16). Although ERp57 could bind directly to TPN and not CRT, we consider this unlikely. ERp57 is known to interact in combination with CRT or CXN with several different proteins besides class I (32–34), whereas TPN is thought to be a class I-specific chaperone (2). Thus, the simplest model consistent with these conclusions is that, during the assembly of the peptide-loading complex, ERp57 binds CRT, and ERp57-CRT only binds to class I-β_m in the presence of TPN.

Interestingly, a recent report by Diedrich et al. (50) used human cells to define an ER complex of ERp57-CXN-TAP-TPN. These authors proposed that, coincident with class I binding, this intermediate complex replaces CXN with CRT to form the peptide-loading complex. However, regardless of the fate of this intermediate, the study by Diedrich et al. (50) is in complete agreement with our report concerning two underlying conclusions: first, that the steady-state association of ERp57 with class I is dependent upon TPN and, second, that when class I is bound, the loading complex includes steady-state levels of CRT and not CXN. This concordance between these two studies indicates that the rules governing the association of ERp57 with the peptide-loading complex are similar for mice and humans. It should be noted that ERp57 can clearly also be detected in association with CXN (32–34).
The fact that we did not detect ERp57 associated with class I assembly intermediates that were not also associated with CRT-TPN-TAP is in apparent conflict with two recent reports (28, 49).

In our approach, we cannot rule out the existence of ERp57-H-chain-β2m complexes lacking TPN. However, such complexes would have to exist transiently and, thus, lack steady-state detection in our assays. In another report, ERp57 association with class I was reported to exist transiently and, thus, lack steady-state detection in our assays. We favor the model that stable association of ERp57 with class I is TPN dependent and correlates precisely with CRT association.

Thus, we favor the model that stable association of ERp57 with class I is TPN dependent and correlates precisely with CRT association.

The fact that we did not detect ERp57 associated with class I may relate to the failure to detect steady-state association of ERp57 with the heavy chain of class I molecules.

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References


10. Ortmann, B., M. Androlewicz, and P. Cresswell. 1994. MHC class I/β2m-TAP and β2m-independent (28). These findings are in conflict with our data and other reports. For example, we report here that five different H chain mutations that disrupt association with TPN-TAP also disrupt ERp57 association with class I. Furthermore, both in our studies with Ld (Fig. 5) and a report by Hughes and Cresswell (26) about HLA-B8, ERp57 association was detected in TPN-positive 221 cells and not TPN-deficient 220 cells. There are also several reports confirming the importance of β2m assembly for H chain association with CRT and TPN-TAP (10–12, 16).

 Thus, we favor the model that stable association of ERp57 with class I is TPN dependent and correlates precisely with CRT association. This timing of ERp57 association with class I, as well as the failure to detect steady-state association of ERp57 with the C1015 H chain mutant, suggests that ERp57 may have class I-related functions other than disulfide bond formation. For example, coincidence association with class I of ERp57 and TPN-TAP may reflect an important role of ERp57 in final peptide trimming or turnover or class I molecules with aberrant peptide loading.

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34, 50), but ERp57 is only detected in association with CRT when class I is also bound.


