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A Region of Tapasin That Affects \( \text{L}^d \) Binding and Assembly

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Tapasin has been shown to stabilize TAP and to link TAP to the MHC class I H chain. Evidence also has been presented that tapasin influences the loading of peptides onto MHC class I. To explore the relationship between the ability of tapasin to bind to TAP and the MHC class I H chain and the ability of tapasin to facilitate class I assembly, we have created novel tapasin mutants and expressed them in 721.220-L\( ^d \) cells. One mutant has a deletion of nine amino acid residues (tapasin \( \Delta 334-342 \)), and the other has amino acid substitutions at positions 334 and 335. In this report we describe the ability of these mutants to interact with \( \text{L}^d \) and their effects on \( \text{L}^d \) surface expression. We found that tapasin \( \Delta 334-342 \) was unable to bind to the \( \text{L}^d \) H chain, and yet it facilitated \( \text{L}^d \) assembly and expression. Tapasin \( \Delta 334-342 \) was able to bind and stabilize TAP, suggesting that TAP stabilization may be important to the assembly of \( \text{L}^d \). Tapasin mutant H334F/H335Y, unlike tapasin \( \Delta 334-342 \), bound to \( \text{L}^d \). Expression of tapasin H334F/H335Y in 721.220-L\( ^d \) reduced the proportion of cell surface open forms of \( \text{L}^d \) and retarded the migration of \( \text{L}^d \) from the endoplasmic reticulum. In total, our results indicate that the 334–342 region of tapasin influences \( \text{L}^d \) assembly and transport.


In the endoplasmic reticulum (ER), the peptide-free MHC class I H chain associates with a multiprotein complex that assists its assembly with peptide and \( \beta_2 \)-microglobulin (\( \beta_2 \text{m} \)). Proteins that compose this complex include tapasin, TAP, calreticulin, and ERP57 (1). The stoichiometry of the complex is such that TAP interacts with four MHC class I-tapasin-calreticulin units (2). Within the complex, tapasin binds directly to the class I H chain (3) and TAP is connected to the class I H chain by tapasin (4–5). Addition of peptide causes a conformational change in the class I H chain and the release of the class I/\( \beta_2 \text{m} \)/peptide heterotrimer from the assembly complex proteins (6–9).

Mutations that have been made in several sites on class I alter the association of class I with tapasin, TAP, and calreticulin. These sites include a loop on the \( \alpha_2 \) domain (10–13), a residue that is glycosylated in the \( \alpha_1 \) domain (9, 13), an area on the \( \alpha_3 \) domain (8–9, 14–16), and residues in the class I cleft (17–19). Because all of these mutations result in loss of association with calreticulin and tapasin, the binding of these two proteins to the class I H chain is likely to be cooperative (13). This cooperativity has complicated the identification of the independent contributions of tapasin and calreticulin to class I assembly.

Most information that can be presumed about the specific role of tapasin has been derived from knockout models. A cellular model that has been amply used is the 721.220 cell line, which is a human B lymphoblastoid cell line with a tapasin defect (4, 20–21). Once believed to express no tapasin, the 721.220 cell line has more recently been found to express, at a very low level, a form of tapasin with a shortened signal peptide and without 49 aa consisting of the N terminus of the mature protein (22). The poor expression of this mutant is due to inefficient ER translocation. The endogenous mutant tapasin in 721.220 cells can bind to TAP, but not to the MHC class I H chain (22).

Examination of class I transfectants of the 721.220 cell line has demonstrated that different transfected human MHC class I allele products have disparate levels of dependence of tapasin for surface expression and Ag presentation, perhaps due to differences in affinity for tapasin or in the availability of stabilizing peptides (21–25). For example, when transfected into 721.220 cells, HLA-A1 and -B8 exhibited severely reduced surface expression and failed to interact with TAP or present Ag to T cells (2, 21). In contrast, HLA-B27 had essentially the same level of surface expression when transfected into 721.220 or 721.220 and tapasin (23). Notably, when expressed on 721.220, the B27 molecules contained a different, less stably bound set of peptides (23). These findings with HLA-B27 are consistent with tapasin exercising a regulatory function in class I binding, perhaps stabilizing MHC class I during de novo peptide binding or during exchange of low- for high-affinity peptides.

Studies on the 721.220 cell line have also contributed to our understanding of tapasin influence on TAP. In 721.220 cells, the binding of peptides to TAP is diminished in comparison with the binding of peptides to TAP in related 721.221 cells, which are tapasin positive (26). A higher steady-state level of TAP is apparent after tapasin transfection of .220 cells, suggesting tapasin may stabilize the TAP heterodimer. TAP stabilization by tapasin is associated with an increase in the quantity of peptide translocated per cell, although the rate of translocation per TAP transporter is not affected (27–28).

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§ Abbreviations used in this paper: ER, endoplasmic reticulum; \( \beta_2 \text{m}, \beta_2 \)-microglobulin; CHAPS, 3-(3-cholamidopropyl)dimethylammonio)-1-propanesulfonate; Endo H, endoglycosidase H.
As an alternative model for studying tapasin function, tapasin knockout mice have been generated recently (29). In these mice, the endogenous H-2Kd and Dd molecules are transported efficiently to the cell surface; however, these molecules are thermally unstable, indicating the absence of stably bound peptides. As a consequence, the steady-state class I cell surface expression is greatly reduced compared with wild type. Furthermore, in these mice, T cell numbers were reduced and the anti-viral CTL response was impaired. Thus, the data derived from studies with these mice suggest tapasin is key to the retention of empty MHC class I, to the surface expression of stable class I, and to T cell development and response.

Overall, postulated functions for tapasin, based on the studies with 721.220 and tapasin−/− mice, are stabilization of TAP, bridging class I to TAP, and quality control of class I peptide loading. Unfortunately, understanding of the molecular mechanisms that underlie these functions is extremely limited. Mutational analysis of the class I H chain cannot contribute much more to this issue than it already has (as described above), due to the cooperativity in binding the class I H chain that is exhibited by calreticulin, TAP, and tapasin. There is very little information on tapasin folding and binding the class I H chain that is exhibited by calreticulin, TAP, and quality control of class I peptide loading.

We have constructed site-directed tapasin mutants and examined their ability to associate with Ld and assist its assembly. The first mutant has a deletion of nine amino acid residues (tapasin Δ334–342), and the second mutant has amino acid substitutions at two positions within the 334–342 region (see Fig. 1). Our findings with tapasin Δ334–342 indicate that it is unable to bind to the Ld H chain, and yet it facilitates Ld assembly and expression nearly as well as wild-type tapasin. Despite its lack of capacity to bind to Ld, we have found that tapasin Δ334–342 was able to bind and stabilize TAP, suggesting that TAP stabilization may play an important role in the positive effect of tapasin on the assembly of Ld. The second mutant (tapasin H334F/H335Y), unlike tapasin Δ334–342, binds to Ld. Expression of tapasin H334F/H335Y in 721.220-Ld results in a decrease in the level of open forms of Ld at the cell surface and a slowed migration of Ld from the ER. Thus, the tapasin H334F/H335Y mutant retains Ld in the cell for an unusually increased proportion of folded Ld at the cell surface. Overall, these data indicate amino acid residues within the tapasin 334–342 region influence tapasin association with Ld.

Materials and Methods

Cell lines
The 721.220 cell line (4, 20–21) originated in the laboratory of Dr. R. DeMars and was graciously provided to us by Dr. T. Spies (Fred Hutchinson Cancer Research Center, Seattle, WA). Due to its N-terminal deletion, the endogenous tapasin present in the 721.220 cells was not detected by the anti-N-terminal tapasin peptide serum (see Materials and Methods) used in some of the experiments described in this manuscript. For transfaction of wild-type and mutant tapasin, 721.220-Ld-RSV.Sneo cells were used as the host cell (Ref. 13; kindly contributed by Dr. T. Hansen, Washington University, St. Louis, MO). The wild-type tapasin cDNA (30) was cloned into pREP10 (a hygromycin-resistant vector; Invitrogen, Carlsbad, CA) and transfected into 721.220-Ld-RSV.Sneo cells as a control. A 721.220-Ld-RSV.Sneo cell line expressing mutant human tapasin with a deletion of the HHSDGSVSL sequence was made (220-Ld Δ334–342); this sequence was chosen because a HHSDGSVSL peptide had been eluted from an HLA-B15 molecule (19, 31). The method used for making the tapasin Δ334–342 mutant was as follows. A human tapasin cDNA (30) was mutagenized using the Quik Change Mutagenesis kit (Stratagene, La Jolla, CA) to remove the sequence encoding residues 334–342 (numbering the tapasin amino acid sequence with +1 as the first amino acid present in the sequence after removal of the leader peptide). Mutant clones were selected and sequenced to confirm the desired deletion and the complete fidelity of the remaining tapasin sequence. Next, the mutant cDNA was cloned into pREP10 next to the vector RSV promoter, and the construct was electroporated into 721.220-Ld-RSV.Sneo cells. Transfectants were selected by resistance to hygromycin as well as G418. The same methods were used to make 220-Ld Δ334–342 + tapasin H334F/H335Y. The TAP-deficient cell lines T2, T2-Ld, T2-Kb, and T2-D2 are generously donated by Dr. T. Hansen, and the cell lines T2-B7 and T2-B27 by Dr. P. Cresswell (Yale University, New Haven, CT).

Antiserum, mAb, and peptides
The mAb 64-3-7 and 30-5-7 were kind gifts from Dr. T. Hansen. The mAb 64-3-7 recognizes the α1 domain of open, peptide-free Ld (32–36), and mAb 30-5-7 recognizes the α2 domain of folded Ld (34–37). The 28–14-8 mAb recognizes the α3 domain of both the open and folded Ld conformations (35, 37). Rabbit anticalreticulin serum (38) was purchased from StressGen Biotechnologies (Victoria, British Columbia, Canada). The rabbit anti-human tapasin Ab directed against an N-terminal tapasin peptide was graciously provided by Dr. T. Hansen. The anti-TAP2 hybridoma 435.3 (39) and the rabbit anti-human tapasin serum (9) have been previously described.

Peptide 334–342 was synthesized by standard solid phase methodologies on an Applied Biosystems (Foster City, CA) model 430A synthesizer. Peptide purification was performed by analytical and preparative HPLC with columns packed with reversed phase silica. The peptide was characterized by amino acid compositional analysis and mass spectrometry.

Immunoprecipitations and Western blots
For immunoprecipitations, the cells were washed three times in PBS containing 20 mM iodoacetamide (Sigma-Aldrich, St. Louis, MO) and lysed in buffer that contained digitonin or 3-[(3-cholamidopropyl)dimethy lammonio]-1-propanesulfonate (CHAPS). The digitonin lysis buffer was 1% digitonin in TBS (pH 7.4) with freshly added 0.2 mM PMSF (Sigma-Aldrich), 0.1 mM N-ethylmaleimide, PMSF, and 20 mM iodoacetamide. The CHAPS lysis buffer was 1% CHAPS (Boehringer Mannheim, Indianapolis, IN) in TBS (pH 7.4) with freshly added 0.1 mM PMSF and 20 mM iodoacetamide. The lysis buffer was supplemented with a saturating volume of mAb before its addition to pelleted cells. After incubation for 1 h on ice, nuclei were removed by centrifugation and lysates were incubated with 3 μg of Anti A-Sepharose beads (Pharmacia, Piscataway, NJ). The beads were washed four times with 0.1% CHAPS in TBS (pH 7.4), and the samples were eluted by boiling in 1× elution buffer (0.125 M Tris (pH 6.8)/2% SDS/12% glycerol/2% bromphenol blue). All immunoprecipitates were electrophoresed on 4–20%, 8%, or 10% acrylamide SDS-PAGE gels (NOVEX, San Diego, CA). After transfer of the immunoprecipitated proteins to Immobilon-P membranes (Millipore, Bedford, MA), Western blots were performed as described (13).

For pulse-chase experiments, 721.220-Ld plus tapasin and 220-Ld plus tapasin H334F/H335Y cells were radiolabeled with 35S-methionine for 30 min. Labeled cells were either washed and lysed immediately, or they were washed and then incubated in medium with 35S nonradioactive methionine for up to 5 h. Following the appropriate chase time, the cells were washed and lysed with 1% CHAPS containing iodoacetamide, PMSF, and 30-5-7 mAb. Lysates were incubated on ice for 50 min and were then centrifuged. The cleared lysates were incubated with protein A-Sepharose beads. The beads were washed extensively, and precipitated protein was eluted from the beads by boiling them in 50 μl of 1× SDS-PAGE buffer for 5 min. Sodium citrate buffer (30 μl of 0.5 M buffer) with 1% 2-ME was added to the eluates. The eluates were divided into two aliquots, one of which was treated with 1 μl of endoglycosidase H (Endo H). All samples were incubated overnight at 37°C, boiled 5 min in 4× elution buffer with 8% 2-ME, and loaded onto Tris-glycine gels. After electrophoresis, proteins on the gels were transferred to Immobilon-P membranes, which were autoradiographed. Transfer of the proteins onto membranes results in quicker detection of the proteins by autoradiography (40). Relative densitometric values were determined with the LAS-1000 imager (Fuji Medical Systems, Stamford, CT).

Flow cytometry
For flow cytometry analysis, cells were suspended at 5×106/ml in PBS with 0.2% BSA and 0.1% sodium azide. Aliquots (0.1 ml each) of the cell suspension were distributed to wells in a 96-well plate. The cells were incubated with saturating concentrations of Anti A-Sepharose beads for 30 min at 4°C, washed twice, and incubated with a fluorescein-conjugated, Fc-specific F(ab′)2, of goat anti-mouse IgG for 30 min at 4°C. The cells were washed twice, resuspended in PBS/BSA/azide, and analyzed on
a FACSCaliber flow cytometer (BD Immunocytometry Systems, San Jose, CA). The CellQuest software was used for statistical analyses. The mean fluorescence value obtained with secondary Ab only was subtracted from each mean fluorescence value obtained with mAb 64-3-7 and mAb 30-5-7 before ratios were calculated. For peptide accessibility assays, the cells were incubated overnight at a density of 1 × 10^6 cells/ml with 250 μM of Ld-binding peptide (IPGLPLSI) (41) in RPMI supplemented with glutamine, penicillin, streptomycin, and FCS. After incubation, the cells were centrifuged and resuspended at 5 × 10^6/ml in PBS with BSA and sodium azide, and flow cytometry assays were performed as described above. For assays to monitor the turnover of class I at the cell surface, cells were treated with brefeldin A at a final concentration of 5 μg/ml for 0, 1, 2, 3, or 4 h. Then the cells were harvested and resuspended at 5 × 10^6/ml in PBS/BSA/sodium azide, and flow cytometry assays were done as described above.

Results and Discussion

The tapasin Δ334–342 mutant is able to bind to TAP, but not to Ld

To analyze tapasin function and MHC class I interaction, we have used a human tapasin mutant (tapasin Δ334–342) that is lacking only nine internal amino acids (Fig. 1). The original rationale for deleting this specific sequence was that this peptide had been eluted from an HLA-B15 molecule found to be associated with tapasin, but was not eluted from two very closely related B15 subtypes that have little or no tapasin association (19, 31). This mutant was transfected into 721.220-Ld cells so that the well-characterized anti-Ld mAb 64-3-7 and 30-5-7 could be used, specific for the open, peptide-free conformation and the folded conformation, respectively (32–37). The open 64-3-7+ Ld conformation, in contrast to the folded 30-5-7+ Ld conformation, is preferentially associated with the ER assembly complex (8–9).

Ld expressed in 721.220 and 721.221 has previously been used for the examination of tapasin function, and the ratio of folded to open Ld expressed by human 721.221 cells was shown to be comparable with that of Ld expressed by mouse L cell fibroblasts (42). This observation indicates that Ld interacts effectively and similarly with either human or mouse tapasin to increase the proportion of Ld expressed in the folded conformation (42). In addition to differences between mouse and human tapasin, another consideration in using Ld expressed in a human cell line is that human β2m has a higher affinity for Ld than does murine β2m (43). Human β2m, therefore, might affect the binding of Ld to individual chaperones (such as tapasin) and/or the overall complex stability differently than murine β2m. However, the resemblance between the surface open:folded ratio for Ld expressed in human 721.221 cells and mouse L cell fibroblasts (42) indicates that, at least at the level of cell surface expression, the ultimate effect of human and mouse β2m on Ld is very similar.

To determine whether tapasin Δ334–342 was able to bind to MHC class I, we immunoprecipitated Ld from .220-Ld, .220-Ld + tapasin, and .220-Ld + tapasin Δ334–342 cells, and probed a Western blot of the immunoprecipitates with antitapasin serum. As shown in Fig. 2, a tapasin band was apparent in the .220-Ld + tapasin lane, but not in the .220-Ld + tapasin Δ334–342 lane.

Thus, the deletion of this small segment of tapasin was sufficient to completely abrogate interaction with Ld. This observation was also made with a separate .220-Ld + tapasin Δ334–342 transfectant (data not shown). We also found that the interaction of calreticulin with the Ld H chain was as poor in .220-Ld + tapasin Δ334–342 as in .220-Ld, although it was strong in the .220-Ld + tapasin control (data not shown). This observation is additional evidence that tapasin Δ334–342 does not bind to Ld, because the binding of normal tapasin to Ld stabilizes Ld/calreticulin interaction.

As mentioned above, our original reason for suspecting that the Δ334–342 tapasin sequence might bind to class I was that the 334–342 peptide had been eluted from an HLA-B15 molecule that interacts with tapasin (19, 31). It has been proposed that tapasin might interact with the groove of the class I H chain (1), and our laboratory and others (17–19) have found that amino acid alterations in the class I groove affect interaction with tapasin and other members of the assembly complex. If the 334–342 peptide binds in the groove of Ld and other MHC class I molecules, this sequence in the context of the whole tapasin molecule might interact with the class I cleft. To determine whether the 334–342 peptide could bind in the groove of many different class I molecules, we tested whether it could induce the surface expression of class I on T2 (expressing endogenous HLA-A2), T2-Ld, T2-B7, T2-B27, T2-Kd, or T2-Dp. Our finding was that this peptide did not significantly induce the surface expression of any of these class I molecules on T2, although known peptide ligands used as controls did induce surface expression (data not shown). Thus, this tapasin sequence, in isolation, does not bind stably in the groove of a wide variety of class I molecules, although our data do not prove that this sequence cannot bind in the groove as part of the whole tapasin molecule.

The tapasin Δ334–342 mutant is able to facilitate Ld surface expression

The tapasin deletion mutant was tested for its ability to promote the expression of folded MHC class I molecules at the cell surface, which is a function of normal tapasin (21). The surface expression of Ld on the .220-Ld, .220-Ld + tapasin, and .220-Ld + tapasin Δ334–342 cell lines was examined by flow cytometry with mAb 64-3-7 and 30-5-7, specific for open and folded Ld, respectively. The ratio of the mean fluorescence obtained with 64-3-7 and 30-5-7 was calculated, and the open/folded surface Ld ratio of .220-Ld + tapasin Δ334–342 was found to be slightly higher than that of .220-Ld + tapasin, but considerably lower than that of .220-Ld (Fig. 3). Thus, the tapasin

FIGURE 1. The location of the in-frame deletion of the HHSDGSVSL sequence and the positions of the H334F/H335Y substitutions are indicated on tapasin.
Δ334–342 mutant was able to assist the expression of folded MHC class I at the cell surface. We also observed a very similar effect on the expression of folded Ld with an independent 220-Ld + tapasin Δ334–342 transfectant (data not shown).

Because 721.220 also expresses a very small amount of endogenous mutant tapasin (22), functions seemingly exhibited by tapasin Δ334–342 in 721.220-Ld cells might conceivably involve complementation between tapasin Δ334–342 and the endogenous mutant tapasin. However, we believe this consideration to be unlikely for the following reason. The amount of available endogenous mutant tapasin in the ER of the 721.220 cell line is very small, due to partial deletion of the signal sequence (22). In contrast, the tapasin Δ334–342 mutant has an intact signal sequence and is expressed under the control of a high-level Ld promoter in 220-Ld + tapasin Δ334–342. Therefore, although a minor degree of complementation cannot be completely excluded, it should not play a major role in the results.

The tapasin Δ334–342 mutant is able to stabilize TAP

Normal tapasin is able to stabilize TAP (27), and we hypothesized that the tapasin Δ334–342 mutant might be facilitating Ld expression by affecting TAP. To test this we examined the ability of the tapasin Δ334–342 mutant to bind and stabilize TAP by immunoprecipitation using Ld lysates of 721.220 cells that were untransfected, transfected with Ld, transfected with Ld and wild-type tapasin, or transfected with Ld and tapasin Δ334–342. As shown in Fig. 4 (top panel), tapasin Δ334–342, like wild-type tapasin, was able to stabilize TAP. This stabilizing activity may result in a larger proportion of secreted Ld in the ER. These data also show that the 334–342 sequence deleted from this mutant is not essential for TAP stabilization, consistent with the region critical for the binding and stabilization of tapasin (27).

The TAP immunoprecipitates were also probed with anti-β2m serum, or transfected with Ld and tapasin Δ334–342. Therefore, although a minor degree of complementation cannot be completely excluded, it should not play a major role in the results.

Tapasin Δ334–342 stabilized TAP and coprecipitated with TAP. TAP was immunoprecipitated from digitonin lysates of 721.220, 721.220-Ld, 721.220-Ld + tapasin, and 721.220-Ld + tapasin Δ334–342 cells with mAb 435.3 (top panel). After electrophoresis on an 8% acrylamide Tris-glycine gel and transfer to a blotting membrane, the immunoprecipitates were probed with anti-tapasin serum (bottom panel). The results shown are representative of those obtained from two separate experiments.
Positions 334 and 335 of tapasin affect Ld transport and surface expression

To begin to identify a particular amino acid residue or residues in the 334–342 region that influence Ld binding and assembly, we generated a site-directed mutant (tapasin H334F/H335Y) that has two substitutions in this region. We expressed this mutant in 721.220-Ld cells and determined that it was expressed at a level equivalent to wild-type tapasin in 721.220-Ld + tapasin (Fig. 5) and that it associated with Ld as well as wild-type tapasin (data not shown). We examined the effect of tapasin H334F/H335Y on the surface expression of Ld by flow cytometry using mAb 64-3-7 and 30-5-7. As shown in Fig. 3, the intracellular presence of tapasin H334F/H335Y caused a decrease in the proportion of open cell surface Ld molecules.

A reduction in the proportion of open surface Ld molecules suggests that relative to wild-type tapasin, the tapasin H334F/H335Y mutant allows the binding of higher affinity peptides to Ld or permits fewer unfolded or poorly folded Ld molecules to escape retention in the ER. If the peptides bound by surface Ld on .220-Ld + tapasin H334F/H335Y are of atypically high affinity, these Ld molecules should be unusually stable at the cell surface. To test this hypothesis, we treated .220-Ld + tapasin H334F/H335Y, along with .220-Ld and .220-Ld + tapasin, with brefeldin A to stop the progression of new Ld molecules from the ER, and we then monitored the expression of surface Ld over time. As shown in Fig. 6, the Ld molecules on .220-Ld + tapasin H334F/H335Y were not detectably more stable than those on .220-Ld plus tapasin.

If the peptides bound by surface Ld on .220-Ld plus tapasin H334F/H335Y are of unusually high affinity, they should be relatively difficult to displace by exogenous peptide ligands. We incubated .220-Ld plus tapasin H334F/H335Y and .220-Ld plus tapasin cells with or without known peptide ligands, assessed their surface Ld expression, and found no significant difference between the inducibility of Ld expressed on .220-Ld + tapasin H334F/H335Y versus 220-Ld + tapasin (data not shown). Thus, the reduction in the proportion of open surface Ld molecules on .220-Ld + tapasin H334F/H335Y seems likely to be due to fewer unfolded or poorly folded Ld molecules escaping ER retention, rather than to the binding of higher affinity peptides.

The presence of a low proportion of open surface Ld molecules might be accompanied by prolonged binding of Ld by tapasin H334F/H335Y in the ER. To test whether the migration of Ld to the cell surface was slowed by tapasin H334F/H335Y, we compared Ld maturation in .220-Ld + tapasin H334F/H335Y and .220-Ld + tapasin by pulse-chase. By 1 h, more Ld molecules were perceptibly susceptible to Endo H in .220-Ld + tapasin H334F/H335Y relative to .220-Ld + tapasin (Fig. 7). Thus, tapasin H334F/H335Y retains Ld within the cell longer than wild-type tapasin.

In summary, we have identified a specific region of tapasin that affects the interaction of tapasin with the Ld H chain. This region of tapasin drew our interest because the 334–342 tapasin sequence was eluted as a peptide from an HLA-B15 subtype that interacts with tapasin, but not from another B15 subtype, differing only at position 116, that does not interact efficiently with tapasin (19, 31). Our finding that the deletion of the 334–342 sequence prevents tapasin from binding to Ld raises the intriguing possibility that tapasin binds within the groove of at least some MHC class I molecules. Similarly, the invariant chain, which regulates MHC class II transport and peptide binding, binds to MHC class II within its groove, and polymorphism in MHC class II seems to influence invariant chain association (47). Furthermore, our analyses of the tapasin Δ334–342 mutant suggests that tapasin stabilization of TAP up-regulates Ld surface expression, perhaps due to provision of additional peptide ligands. In addition, our results with tapasin H334F/H335Y suggest that small changes in the amino acid sequence of tapasin can increase the length of intracellular retention of MHC class I, and, accordingly, affect the proportion of folded MHC class I molecules at the cell surface. These observations

FIGURE 5. Tapasin H334F/H335Y and wild-type tapasin were expressed at similar levels. Cells (721.220, 721.220-Ld, 721.220-Ld + tapasin, and 721.220-Ld + H334F/H335Y) were harvested, washed, lysed, and centrifuged. Supernatants were removed and boiled, and aliquots were electrophoresed on a 10% acrylamide Tris-glycine gel. Proteins were transferred from the gel to a blot membrane, and the membrane was probed with anti-N-terminal tapasin serum. The results shown are representative of those obtained in three separate experiments.

FIGURE 6. Tapasin H334F/H335Y and wild-type tapasin cause similar cell surface Ld stability. Cells (721.220-Ld + tapasin, 721.220-Ld + tapasin H334F/H335Y, and 721.220-Ld) were cultured in the presence of brefeldin A at a final concentration of 5 μg/ml for 0, 1, or 3 h, and then were tested by flow cytometry with mAb 30-5-7 and 28-14-8. The percentage of 30-5-7+ (top panel) and 28-14-8+ (bottom panel) Ld remaining at each time point was calculated and is shown in the graphs.
enhance our comprehension of the functional domains of tapasin and indicate that manipulation of tapasin sequence can affect MHC class I egress and cell surface conformation.

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