The mechanism of action of tapasin in the peptide exchange on MHC class I molecules determined from kinetics simulation studies

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To understand the mechanism of action of the chaperone protein tapasin, which mediates loading of high-affinity peptides onto major histocompatibility complex (MHC) class I molecules in the antiviral immune response, we have performed numerical simulations of the class I-peptide binding process with four different mechanistic hypotheses from the literature, and tested our predictions by laboratory experiments. We find – in agreement of experimental and theoretical studies – that class I-peptide binding in cells is generally under kinetic control, and that tapasin introduces partial thermodynamic control to the process by competing with peptide for binding to class I. Based on our results, we suggest further experimental directions.

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1. Introduction

MHC class I molecules play a central role in the mammalian antiviral immune response. They consist of a transmembrane heavy chain and a light chain, beta-2 microglobulin (β2m), which associate in the lumen of the endoplasmic reticulum (ER) after their synthesis. Class I molecules select their ligand from the peptides that are generated by the proteasome in the cytosol and transported into the lumen of the endoplasmic reticulum by the transporter associated with antigen presentation (TAP) (Uebel and Tampe, 1999). Out of a pool of peptides of different lengths and sequences, class I molecules bind those that match their canonical binding motifs and optimum length (usually 8–10 amino acids) and then proceed to the surface of the cell, where the complexes of class I dimers and peptides are scrutinized by cytotoxic T lymphocytes (CTL) through their T cell receptors (TCRs) in conjunction with CD8 molecules (Fig. 1). Circulating CTL do not normally react to cell-endogenous (self) peptides, and the binding of a TCR to a peptide-class I complex usually leads to the induced apoptosis of the presenting cell. In this way, the immune system surveys the intracellular proteome of all nucleated cells and eliminates those cells that, because of a viral infection or a malignant mutation, harbor novel proteins.

Among the chaperone proteins that support the maturation of class I molecules in the ER, the transmembrane protein tapasin holds a special place (Sadasivan et al., 1996). Most class I allotypes need it to form tight complexes with peptides of sufficient affinity so they can pass ER quality control and exit to the cell surface (Elliott and Williams, 2005). The mechanism by which tapasin supports the selection of high-affinity peptides of appropriate length and sequence in the presence of an excess of low-affinity peptides has been subject to much research and speculation, and a number of different models for tapasin function have been proposed (Wright et al., 2004). One ingenious experiment followed the thermal stability of cohorts of peptide-class I complexes by pulse chase in live cells and demonstrated that tapasin gradually increases the average affinity of the pool of peptides bound to tapasin-dependent class I molecules over time (Williams et al., 2002). The action of tapasin on peptide binding is most likely the result of direct interactions between tapasin and the class I molecule. This is supported by evidence from coimmunoprecipitations that show a tapasin requirement for class I association with TAP and other chaperones in the peptide loading complex (PLC) (Ortmann et al., 1997) and from the identification of residues both in class I and in tapasin that are essential for this interaction (Bangia et al., 2002).
Fig. 1. MHC class I mediated antigen presentation. MHC class I molecules assemble in the ER from the heavy chain (HC) and beta-2 microglobulin (β2m) and bind peptides that are generated in the cytosol by the proteasome and transported into the ER lumen by the TAP transporter. Peptide binding is assisted by tapasin, which binds directly to class I, together with the other members of the peptide loading complex, ERp57 and calreticulin (CRT). Suboptimal (low affinity) peptides are depicted by green triangles, and high-affinity peptides by a blue pentagon. Following peptide binding, class I molecules leave tapasin behind and travel to the cell surface where they present the bound peptide to killer T cells.

et al., 1999; Lewis et al., 1996; Peace-Breuer et al., 1996; Turnquist et al., 2002). Indeed, tapasin binds close to the peptide binding groove, next to the small α2-1 helix, the position and mobility of which are strongly influenced by the presence of peptide (Elliott, 1997; Zacharias and Springer, 2004). By manipulating the position of this helix, tapasin has been proposed to control peptide association with class I independently of the peptide sequence (Wright et al., 2004). This assertion is supported, but not proven, by the recent crystal structure of tapasin and the modeling of a tapasin-class I complex (Dong et al., 2009).

Some researchers have suggested that the gradual increase of peptide affinity to class I molecules occurs because tapasin prevents the dissociation of preformed class I-peptide complexes (Zarling et al., 2003), enabling complexes to travel to the cell surface that would otherwise fall apart. Since tapasin itself is not found at the plasma membrane, such a stabilization would have to include an irreversible conformational change in class I to ‘lock in’ the peptides; but so far, differences in class I conformation between tapasin-positive and -negative cells, or between tapasin-dependent and -independent class I molecules, have not been detected (Peaper and Cresswell, 2008).

It is reasonable to assume that tapasin influences the kinetics or thermodynamics of the peptide binding process by binding to the peptide-free, the peptide-bound, or an intermediate form of the class I molecule, stabilizing or destabilizing it and thus shifting the equilibrium of the binding reaction, or influencing its component rates. Such influence has been suggested to be purely catalytic, such that peptide binding is made more efficient by the stabilization of an intermediate of the binding reaction (Sieker et al., 2007). This scenario appears to solve a conceptual paradox: the dissociation half-times even of low-affinity peptides are longer than the time class I takes for transit through the ER, leading a reviewer to propose that ‘time is running faster inside the ER’ (Elliott and Williams, 2005). If peptide binding and dissociation was catalyzed by tapasin, then class I molecules could probe more peptides while in the ER, and consequently acquire higher-affinity ones more reliably. Alternatively, tapasin may transiently stabilize either the peptide-free form (competing with peptide) or the peptide-bound form (accelerating the binding of peptide but not its dissociation). Competition of tapasin with peptide would decrease the affinity of class I towards all peptides and accelerate peptide dissociation (but not association) (Wright et al., 2004).

To differentiate between hypotheses that have arisen from experiments, numerical network simulations have proven highly useful (Bornheimer et al., 2004; Calzone et al., 2007; Salazar et al., 2008; Stites et al., 2007). If a group of processes such as chemical reactions or ligand–receptor interactions can be cast into a reaction scheme that leads to a set of ordinary differential equations (ODEs), i.e., a numerical model, and experimental data are available for some of the parameters, then numerical simulations can be used to suggest values for the remaining parameters, and to determine the consistency and explanatory power of the hypotheses.

We present here a numerical model for peptide binding to class I in live cells, based on experimental data, with which we

have performed a comprehensive set of mathematical simulations to test the current hypotheses for tapasin action. We find that only the peptide competition hypothesis, which assumes competition between class I and tapasin for peptide binding, can explain the experimental observations. This hypothesis is validated by a tapasin supertransfection experiment, which we carry out to test its predictions. We suggest further laboratory experiments to determine the parameters of the model.

2. Materials and methods

Kinetic modeling was carried out in three steps: Establishment of a reaction scheme, mathematical formulation, and computational implementation.

2.1. Establishment of a basic biochemical reaction scheme in the absence and presence of tapasin

The derivation of the biochemical reaction scheme without (Fig. 2A) and with tapasin (Fig. 3A) is described in Section 3. The species are: P₁ and P₂, peptides 1 (high affinity) and 2 (low affinity); C, MHC class I molecule (heterodimer of heavy chain and β₂m); T, tapasin; and their combinations (e.g., TCP₁ is the complex of tapasin, class I, and peptide 1). X is the class I species that has exited from the reaction compartment, towards the trans-Golgi and the cell surface. Rate constants are named such that \( k_{a,b} \) is the rate constant of the reaction that leads from species a to species b.

2.2. Mathematical formulation

The reactions of the network were described with mass action kinetics. For example, the rate of binding of the peptide, P₁, to the class I molecule, C, is

\[
\text{Rate} = k_{C,CP1}[C][P1] - k_{CP1,C}[CP1]
\]

All reaction rates are listed in Table 1. The reaction rates were used to describe the network with a system of six ODEs (Table 2). Free \([P₁]\) and \([P₂]\) and the overall tapasin concentration \([T]₀ = [TCP₁] + [TCP₂] + [TC] + [T]) were kept constant throughout.

Table 1

<table>
<thead>
<tr>
<th>Nr.</th>
<th>Reaction</th>
<th>Rate</th>
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<tbody>
<tr>
<td>R1</td>
<td>P₁ binding to C</td>
<td>(k_{C,CP1}[C][P1] - k_{CP1,C}[CP1])</td>
</tr>
<tr>
<td>R2</td>
<td>P₂ binding to C</td>
<td>(k_{C,CP2}[C][P2] - k_{CP2,C}[CP2])</td>
</tr>
<tr>
<td>R3</td>
<td>T binding to C</td>
<td>(k_{C,TC}[C][T] - k_{TC,C}[TC])</td>
</tr>
<tr>
<td>R4</td>
<td>P₁ binding to TC</td>
<td>(k_{C,TP1}[TC][P1] - k_{TP1,TC}[TCP1])</td>
</tr>
<tr>
<td>R5</td>
<td>P₂ binding to TC</td>
<td>(k_{C,TP2}[TC][P2] - k_{TP2,TC}[TCP2])</td>
</tr>
<tr>
<td>R6</td>
<td>T binding to CP₁</td>
<td>(k_{C,TP1}[CP1][T] - k_{TP1,CP1}[TCP1])</td>
</tr>
<tr>
<td>R7</td>
<td>T binding to CP₂</td>
<td>(k_{C,TP2}[CP2][T] - k_{TP2,CP2}[TCP2])</td>
</tr>
<tr>
<td>R8</td>
<td>Export of CP₁</td>
<td>(k_{C,TP1}[CP1])</td>
</tr>
<tr>
<td>R9</td>
<td>Synthesis of C</td>
<td>(k_{C})</td>
</tr>
<tr>
<td>R10</td>
<td>Degradation of C</td>
<td>(k_{C,deg}[C])</td>
</tr>
</tbody>
</table>
Fig. 3. Comparison of the suggested models for tapasin action. (A) Implementation of the different hypotheses for tapasin (T) action into the reaction network. Open arrows represent rates that are amplified by the acceleration factor, f. (B–D) Comparison of the models for f = 100. The parameters of tapasin binding (tapasin on-rates, off-rates, and [T]) were varied and the resulting values for export efficiency and average dwelling time plotted. The tapasin association rates (kT-on) are kC, TCP1, and kCP2; the tapasin dissociation rates (kT-off) are kTC, TCP1, and kTCP2. In the graphs, kT-on and kT-off on the x-axis correspond to the values before multiplication by f, where required by the model. The arrows mark the value of the x-axis parameter that was fixed in the simulations that varied the other two parameters. ADT: average dwelling time. An overview of the entire parameter space is shown in Fig. S2.

Table 2

<table>
<thead>
<tr>
<th>Differential equations. Equations in italics are valid only for the minimal model that does not take tapasin into account.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimal model without tapasin:</td>
</tr>
</tbody>
</table>
| \[
\frac{d[C]}{dt} = R_9 - R_1 - R_2 \quad \text{(if no tapasin)}
\]
| \[
\frac{d[CP_1]}{dt} = R_1 - R_8 \quad \text{(if no tapasin)}
\]
| \[
\frac{d[CP_2]}{dt} = R_2 \quad \text{(if no tapasin)}
\]
| Complete model with tapasin: |
| \[
\frac{d[C]}{dt} = R_9 - R_1 - R_2 - R_3 - R_10 \]
| \[
\frac{d[CP_1]}{dt} = R_1 - R_6 - R_8 \]
| \[
\frac{d[CP_2]}{dt} = R_2 - R_7 \]
| \[
\frac{d[TC]}{dt} = R_3 - R_4 - R_5
\]
| \[
\frac{d[TCP_1]}{dt} = R_4 + R_6
\]
| \[
\frac{d[TCP_2]}{dt} = R_5 + R_7
\]

2.3. Computational implementation

The ODE system was implemented in Mathematica 6.0.1. Steady-state solutions were found analytically. The export efficiency (percentage of MHC class I molecules that leave the reaction compartment towards X) was calculated by

\[
\text{Export efficiency} = \frac{k_{CP_1,X}[CP_1]_{\text{steady-state}}}{k_{CP_1,X}[CP_1]_{\text{steady-state}} + k_{C,\text{Deg}}[C]_{\text{steady-state}}}
\]

The average dwelling time (ADT) was defined as the time after which half of a cohort of freshly synthesized MHC class I molecules have been exported from the reaction compartment.
under steady-state conditions, equivalent to the half-time of acquisition of endo-β-N-acetylglucosidase H (Endo H) resistance in a laboratory pulse-chase experiment. The ADT was determined by numerical simulation, with starting values given by the steady-state concentrations, as described in the supplementary methods. When varying reaction rate parameters, we considered that according to the second law of thermodynamics, the overall $K_D$ of any reaction in the system must be independent of the chosen path (i.e., $K_D(a,b) = K_D(a,c,b) = K_D(a,d,b)$).

2.4. Definitions

In the context of this work, we define: ‘scheme’ or ‘network’, a biochemical reaction network with nodes (species) and edges (reactions); ‘numerical model’, a set of ODEs based on the scheme; ‘hypothesis’, a proposal for specific parameter sets in the numerical model, which explains experimental data. For example, ‘peptide competition hypothesis’ refers to a mechanistic suggestion for the action of tapasin from the experimental literature, while ‘peptide competition model’ refers to the numerical model with a set of parameters that is derived from the peptide competition hypothesis.

2.5. Cells, transfections, and fluorescence microscopy

Mouse ear fibroblasts (MEF) were a kind gift from Luc van Kaer (Vanderbilt University). The tapasin expression construct was a kind gift from Frank Momburg (German Cancer Research Center). The H-2Db-GFP construct, peptide and DNA electroporation, and fluorescence microscopy procedures are described in Garstka et al. (2007).

3. Results

3.1. A simple numerical model for peptide binding to class I molecules

In order to estimate some parameters from the published data, we initially designed a minimal reaction scheme (Fig. 2) for the binding of two different peptides to class I under cellular conditions, i.e., including synthesis, degradation, and surface transport of class I. In Fig. 2A, species C is peptide-free class I, which is synthesized at a constant rate $k_C$ and degraded in a first-order reaction ($k_{C,Deg}$). It forms complexes (CP1 and CP2) with either of two peptides, P1 and P2, which differ in their dissociation constants ($K_D$). P1 being a high-affinity peptide with a smaller $K_D$ than the low-affinity peptide, P2. P1 and P2 represent cohorts of peptides with such properties at the extremes of a continuum of binding affinities. In the cell, only class I molecules with high-affinity peptides traffic to the cell surface, while low-affinity peptide complexes (CP2) are retained in a compartment prior to the median Golgi (Garstka et al., 2007) and, by recycling to the ER, given another possibility to bind high-affinity peptide. Therefore, in our scheme, the only exit (X) from the reaction compartment is from the complex with the high-affinity peptide, CP1.

To generate a numerical model, we described the reactions in Fig. 2A with mass action kinetics (Table 1, reactions in italics) and derived a system of differential equations (Table 2, italics). For the assignment of values to the reaction rate constants, we used data from the experimental literature (a selection is summarized in Table S1). According to most measurements, peptide dissociation rates ($k_{CP1,C}$ and $k_{CP2,C}$), which are strongly temperature dependent, are approximately $1 - 5 \times 10^{-2}$ s$^{-1}$ for nonameric peptides at room temperature, which corresponds to a dissociation half-time of about 20 h, and around $1 - 4 \times 10^{-2}$ s$^{-1}$ for longer peptides. The peptide-class I association rates ($k_{ACP1}$ and $k_{ACP2}$ in our scheme) are more difficult to measure, and the differing values in the literature are likely due to the use of different methods such as fluorescence spectroscopy and immunoprecipitation, which may monitor different molecular events on the reaction pathway. In a study that compared association rates of high- and low-affinity peptides, the association rate to the murine class I molecule, H-2D$^b$, of the low-affinity peptide, YSNENMDAMESSTL, was five times slower than that of the high-affinity ligand, YSNENMDAM (Springer et al., 1998). Finally, the measured values of the $K_D$, which is the ratio between the dissociation and association rate constants, are remarkably similar between studies and class I allotypes, usually in the range of 10–100 nM. Longer and shorter peptides have significantly increased $K_D$s. The upper limit for a T cell epitope was suggested to be 500 nM (Sette et al., 1994).

Based on the published data, we defined for the high-affinity peptide, P1, an association constant ($k_{CP1,C}$) of $2 \times 10^{2}$ M$^{-1}$ s$^{-1}$ and a dissociation rate ($k_{CP1,C}$) of $2 \times 10^{-2}$ s$^{-1}$, which results in a $K_D$ of 10 nM. For the low-affinity peptide, P2, we defined a five-fold lower association constant ($k_{CP2,C}$) of $4 \times 10^{4}$ M$^{-1}$ s$^{-1}$ and a dissociation rate ($k_{CP2,C}$) of $0.4$ s$^{-1}$, which gives a $K_D$ of 10 μM. In our simulation, P1 represents a diverse cohort of high-affinity peptides. Its concentration, according to a published estimate (Yewdell et al., 2003), exceeds that of P1 by a factor of 100. Since we represented peptide binding as a one-step reaction, we chose our parameters such that the equilibrium dissociation constant corresponded to the ratio of the kinetic dissociation and association constants; this led to higher dissociation rate constants than those in some publications, but the ratio between the rate constants for high- and low-affinity peptides was preserved.

3.2. Refining the parameter space of the simple numerical model

On the basis of these experimentally determined parameters, we approximated by simulation the remaining parameters of the simple reaction scheme, namely the synthesis rate $k_C$, the degradation rate $k_{C,Deg}$, the trafficking export rate of the CP1 complex $k_{CP1,X}$, and the concentration of P1, which in turn determines the concentration of P2. We made educated guesses of the value ranges, based on experimental data wherever possible, and performed simulations over these value ranges for all parameters to observe the behavior of the system. We used two experimental evaluations to observe our simulation data. First, once class I molecules are synthesized, they remain in the peptide binding compartment (ER/cis-Golgi) for between 20 and 80 min, after which they become resistant to endoglycosidase H, a hallmark for arrival in the medial Golgi (Williams et al., 1985). We therefore monitored, in our simulations, the average time that a cohort of class I molecules needed from synthesis to arrival at the exit species, X, and termed it the ‘average dwelling time’ (ADT; see the supplementary methods). Second, at steady state, about 150 class I molecules per minute leave the ER to go to the cell surface (Porgador et al., 1997). We monitored both the number of class I molecules exiting the reaction compartment per minute (the flux CP1 → X) and the ratio of this number to that of class I molecules leaving the system by any route (i.e., towards X plus degradation). This latter number, which is zero for total degradation and 1 for total export of class I, we called the ‘export efficiency’. The complete results of our multi-dimensional simulations are in Fig. S1; Fig. 2B–E shows the variation of individual parameters against a constant background.

To find a value range for the degradation rate, $k_{C,Deg}$, we considered the observation that class I molecules that fail to bind peptides are degraded in the ER with a half-life of about 1 h (Kienast et al., 1998), which corresponds to a $k_{C,Deg}$ of $2 \times 10^{4}$ s$^{-1}$. We therefore assumed $k_{C,Deg}$ to be between $10^{-6}$ s$^{-1}$ and $10^{-2}$ s$^{-1}$. In our simulations, a clear correlation of both export efficiency and average dwelling time with $k_{C,Deg}$ was observed. $k_{C,Deg}$ values between approximately $3 \times 10^{-5}$ s$^{-1}$ and $10^{-3}$ s$^{-1}$ resulted in a range of
average dwelling times between 20 and 80 min (Fig. 2B). We fixed \( k_{\text{Deg}} \) to \( 3 \times 10^{-4} \, \text{s}^{-1} \), in good agreement with the experiment.

Intracellular peptide concentrations were suggested from the experiment to lie between \( 2 \times 10^{-7} \, \text{M} \) and \( 1.6 \times 10^{-5} \, \text{M} \) (Yewdell et al., 2003). It is unclear to what extent the concentration in the ER lumen differs from this value, given the ATP-driven import of peptides by TAP (which might lead to an enrichment). We tested a wide range of \( P_1 \) concentrations, from \( 10^{-11} \, \text{M} \) to \( 10^{-6} \, \text{M} \), which corresponds to a total peptide concentration (including \( P_2 \), with \( [P_2] = 100 \times [P_1] \)) of \( 10^{-9} \, \text{M} \) to \( 10^{-4} \, \text{M} \). In our simulations, export efficiency increased strongly with the peptide concentration, suggesting that the export of high-affinity class I-peptide complexes in the model is promoted by an increase of the peptide concentration in the ER. Similar observations have been made in vivo (Day et al., 1997; Garstka et al., 2007). We fixed the total peptide concentration at \( 10^{-7} \, \text{M} \), which corresponds to \( [P_1] = 10^{-8} \, \text{M} \), where the export efficiency is approximately 0.3 (Fig. 2C). This mirrors the behavior of moderately tapasin-dependent class I molecules such as HLA-B8 (Williams et al., 2002), which is exported to the cell surface to some extent even in the absence of tapasin.

\( k_{\text{CP1,tx}} \) is the export rate constant of high-affinity class I-peptide complexes to the cell surface. An estimate of this number was given by Yewdell et al. (2003), who noted that the half-life of class I at the cell surface is about 10 h (corresponding to a surface degradation rate constant of \( 1.9 \times 10^{-5} \, \text{s}^{-1} \)). Since roughly 50% of cellular class I molecules are present at the cell surface, the rate constant of the transport to the cell surface must be equal to or larger than this number. Therefore, we decided to sample \( k_{\text{CP1,tx}} \) in a range from \( 2 \times 10^{-5} \, \text{s}^{-1} \) upwards. As expected, the ADT decreased with increasing \( k_{\text{CP1,tx}} \), but export efficiency increased (Fig. 2D).

The synthesis rate of class I, \( k_c \), needs to be large enough to replace the 150 molecules that travel to the surface each minute (see above), accounting for ER degradation. We used a value of 250 fl for the volume of the ER of a single cell (Griffiths et al., 1984) and a scaling coefficient of 0.3 to correct for the membrane-bound nature of class I (see the supplementary text). Then, a \( k_c \) of \( 10^{-8} \, \text{Ms}^{-1} \) would correspond to about 45 MHC class I molecules synthesized per second, or 2700 molecules/min. In our simulations, variation of \( k_c \) from \( 10^{-10} \) to \( 10^{-6} \, \text{Ms}^{-1} \) had no effect on either export efficiency or average dwelling time (Figs. 2E and S1), and we set \( k_c \) to \( 10^{-9} \, \text{Ms}^{-1} \) for the subsequent simulations.

#### 3.3. Implementing the different hypotheses of tapasin function

We next extended our reaction scheme to include tapasin (T; Fig. 3A), with the assumption that one class I molecule binds to one molecule of tapasin (Bangia and Cresswell, 2005). Tapasin is displaced from class I by high-affinity peptide (Ortmann et al., 1994; Suh et al., 1994; Wright et al., 2004), but it may bind to a complex of class I and low-affinity peptide. To obtain a complete reaction scheme (Monod et al., 1965), we permitted all forms of class I to bind to tapasin. Compared to the simple scheme, the complete scheme has three additional sets of parameters: the overall tapasin concentration \( [T]_0 \) (i.e., the sum of concentrations of all tapasin-containing species, \( [T] + [TC] + [TCP_1] + [TCP_2] \)), the tapasin association \( (k_{\text{TCP1,TCP1}}, k_{\text{TCP1,TC}}, k_{\text{TCP2,TC}}) \) and dissociation \( k_{\text{TCP1,TCP1}}, k_{\text{TCP1,TC}}, k_{\text{TCP2,TC}} \) rate constants of the transition state (\( \Delta G^\circ \) of the peptide binding reaction, accelerating both forward and reverse reaction, but not affecting the equilibrium constants. This peptide exchange catalysis hypothesis was implemented in our scheme by increasing both the association and dissociation rates of peptides with tapasin-associated MHC class I (\( k_{\text{TCP1,TC}}, k_{\text{TCP2,TC}} \)) by the acceleration factor, \( f \).

A fourth hypothesis is based on the results of molecular dynamics simulations of class I molecules (Sieker et al., 2007). It suggests that peptide editing occurs because tapasin acts like an enzyme to catalyze the transition between the peptide-free and peptide-occupied states of class I by decreasing the reaction free energy of the transition state (\( \Delta G^\circ \)) of the peptide binding reaction, accelerating both forward and reverse reaction, but not affecting the equilibrium constants. This peptide exchange catalysis hypothesis was implemented in our scheme by increasing both the association and dissociation rates of peptides with tapasin-associated MHC class I (\( k_{\text{TCP1,TC}}, k_{\text{TCP2,TC}} \)) by the acceleration factor, \( f \).

Instead of increasing the rate constant of some reactions by the factor \( f \) to implement our models, we also tested the approach of slowing the reverse reaction by the same factor. This did not lead to a significantly different behavior of the system (data not shown). Some restrictions on the implementation of the models were imposed by the circular nature of the reaction network (see Section 2). Table 3 gives an overview of the parameters in the simulation of the simple and the extended reaction scheme.

#### 3.4. Comparison of the hypotheses

We next compared the four hypotheses in multi-dimensional simulations with initial educated guesses for the value ranges of the acceleration factor \( f \), the total tapasin concentration \( [T]_0 \), and the association and dissociation rates of tapasin. We observed the ADT and the export efficiency, which were 30 min and 0.28, respectively, without tapasin. An increase in the export efficiency signifies successful peptide editing by tapasin, while the ADT should not be significantly affected by peptide editing. Panels B–D of Fig. 3 show the results for the variation of one parameter at a time, while the full set of data is shown in Fig. S2.

The total concentration of tapasin \( [T]_0 \) is likely to be in the same order of magnitude as the concentration of class I (approximately...
1–10 μM (Yewdell et al., 2003). We varied [T]₀ between 10⁻⁷ M and 10⁻¹ M. At concentrations above 10⁻⁵ M, ADTs increased, presumably because class I molecules formed tight complexes with tapasin and could not exit the reaction compartment (Fig. 3D). At any concentration between 10⁻⁷ M and 10⁻³ M, differences in export efficiency were visible between the hypotheses. We thus used 10⁻⁵ M for [T]₀.

The three association and three dissociation rate constants of tapasin (summarized below as k₅₄₈ and k₅₃₉) determine the affinity of tapasin towards class I. In vitro studies with truncated soluble proteins, this interaction was very weak (Chen and Bouvier, 2007), but in vivo, it is likely to be stronger, since both molecules are confined to the membrane and bind cooperatively in the context of the PLC. We decided to test k₅₃₉ and k₅₄₈ between 10⁻¹ and 10⁵ (M⁻¹ s⁻¹ and s⁻¹, respectively) (Fig. 3B and C). Since different class I allotypes bind very different peptides, it is unlikely that tapasin itself interacts with the peptide to distinguish between high- and low-affinity ligands. Therefore, in the binding-only model, all three k₅₄₈ were assumed to be identical, and likewise all three k₅₃₉. Individual association or dissociation rate constants were multiplied by the acceleration factor, f, where required by the model. Even though we tested f between 1 (no effect on class I-peptide affinity) and 10⁶ (Fig. S2), our value of f = 100 was based on the experimental results of Bouvier and co-workers, who found a 100-fold increase in the dissociation rate of some peptides in the presence of tapasin (Chen and Bouvier, 2007). An effect of tapasin on the export efficiency was only observed if the k₅₄₈ were at least 1000 times higher than the k₅₃₉, corresponding (at [T]₀ = 10⁻⁵ M) to a [TC]/[TC₅₉] ratio of 1/100. Thus, at least one out of 100 class I molecules needed to be associated with tapasin at steady state for a peptide editing effect to become visible. With an acceleration factor f of 100, the actual values of k₅₄₈ and k₅₃₉ did not influence the export efficiency, as long as the k₅₃₉/k₅₄₈ ratio was fixed. We fixed k₅₄₈ at 1 s⁻¹ and k₅₃₉ at 10⁴ M⁻¹ s⁻¹.

The parameter values that were established through these comprehensive simulations are pointed out by the arrows in Fig. 3B–D. Under these conditions, the implementations of the four hypotheses behave remarkably differently. Peptide competition, complex stabilization, and catalysis models all lead to some peptide editing (an increase in the export efficiency above 0.28), with the complex stabilization and catalysis models showing a much stronger effect. On the other hand, the complex stabilization and especially peptide competition model show a significant increase in the ADT by retaining class I molecules in the binding compartment. We showed that these results are remarkably robust towards changes in the parameters (see the supplementary text).

### 3.5. Tapasin overproduction leads to decrease in surface class I

To further differentiate between the four hypotheses in a laboratory experiment, we decided to assess the effect of an increase in the amount of tapasin on the distribution of class I between the cell surface and the interior. The surface/interior ratio of class I, which is observable by microscopy, can be calculated from our model since at steady state, the flow of class I molecules to the surface (CP₁ → X) is equal to the flow from the surface into the degradation compartment (endosomes and lysosomes), and the decay half-time of class I molecules at the cell surface is known to be between 5 and 48 h (see the supplementary methods). Fig. 4A shows that the binding-only and the catalysis model predict only moderate changes to the surface/interior ratio of class I with a tenfold increase in [T]₀, whereas the complex stabilization and especially the peptide competition model predict a drastic decrease of this ratio. For the peptide competition model, the ratio dropped from 2.6 (at 10⁻⁵ M tapasin) to 0.4 (at 10⁻⁴ M), suggesting that at an elevated concentration of tapasin, the majority of class I might become retained in the cell. To test this prediction in practice, we transfected mouse ear fibroblasts (which contain endogenous tapasin) with a fusion of the mouse class I molecule, H-2Dk, to the Green Fluorescent Protein (GFP), and imaged the intracellular distribution of the Dk-GFP fusion by confocal fluorescence microscopy. We found that the protein was in the ER and at the cell surface (visible especially well at the cell edges, see the arrow in Fig. 4B). Intriguingly, when the Dk-GFP expressing cells were supertransfected with tapasin, the class I fluorescence became limited to the cell interior (Fig. 4B and C). In an analogous experiment, we used CHO cells, where murine class I molecules can be moved to the cell surface by the electroporation of specific peptides (they are normally retained in the ER, presumably because of inefficient loading with endogenous peptides (Garstka et al., 2007)). This cell surface transport is again suppressed by the presence of supertransfected tapasin (Fig. 4 D and E). Our experimental results point to an intracellular retention of class I in the presence of elevated levels of tapasin, and they support the peptide competition and complex stabilization hypotheses.

### 4. Discussion

After their synthesis in the ER, class I molecules bind low-affinity peptides before selecting a high-affinity peptide (Williams et al., 2002). Since the transit time through the ER is in the same order as the dissociation times of many low-affinity peptides, namely tens of minutes, there is no time to reach a thermodynamic equilibrium of all possible peptide-class I species (Elliott and Williams, 2005). Thus, peptide editing occurs under the conditions of a kinetically controlled steady-state. This is mirrored in our simulations: instead of becoming almost quantitatively bound to the high-affinity peptide (as would be expected under equilibrium conditions, see the supplementary text), most class I molecules are degraded when tapasin is not present, with only 28% leaving the reaction compartment towards the cell surface. Under these circumstances, any modification that increases the degree of thermodynamic control of the peptide binding process will lead to peptide editing.

The simplest way in which this can be achieved is if class I molecules are retained in the reaction compartment such that they can bind and dissociate more peptides in order to find a high-affinity ligand. This seems to be achieved, to a small extent, in the binding-only model, but only if the k₅₄₈/k₅₃₉ ratio is sufficiently high (Fig. 3B and C). However, this kind of editing mechanism brings about a predictable increase in the ADT.

The remaining three models introduce thermodynamic control by an increase in the rate of association (complex stabilization model) or dissociation of peptides (peptide competition model), or...
Fig. 4. Tapasin overproduction decreases class I surface expression in the numerical model and in the experiment. (A) The peptide competition and complex stabilization models predict a strong decrease of surface class I upon tapasin overexpression. Cell surface to cell interior ratios of class I at steady-state were calculated for each model, as described in the text, for a tapasin concentration of $10^{-5}$ M (as used in all simulations described above, white bars) and $10^{-4}$ M (representing 10-fold overexpression of tapasin, black bars and label ‘+ tpn’). The error bars denote the anticipated experimental standard error of 15%. (B) Mouse ear fibroblasts were transfected with H-2D$^b$-GFP (top row) or H-2D$^b$-GFP and tapasin (tpn, bottom row). Surface expression of class I (arrow) is visible in the absence but not in the presence of overexpressed tapasin. Scale bar, 50 μm. (C) Quantification of B. Individual cells ($n = 245, 158$) were scored for surface expression or no surface expression. The white bar shows untransfected cells expressing natural amounts of tapasin, the black bar (label ‘+tpn’) shows cells transfected with tapasin. The error bars denote the SEM. (D) Chinese hamster ovary (CHO) cells were electroporated with a plasmid encoding H-2D$^b$-GFP and with FAPGNYPAL peptide and a plasmid encoding tapasin in the combinations indicated. In the cells electroporated with H-2D$^b$-GFP and peptide, surface expression of class I is visible (arrow); it is suppressed by simultaneous introduction of tapasin. Scale bar, 10 μm. (E) Quantification of D, done as in C ($n = 198, 210, 202, 200$). The white bar shows the cells with peptide but without tapasin.

both (catalysis model). All three changes bring about faster peptide exchange and thus allow more class I molecules to bind high-affinity peptides and escape degradation. Still, the three models make different predictions that can be used to discriminate them. The complex stabilization model predicts cooperativity between the binding of peptide and tapasin, which contradicts many experiments that show displacement of tapasin from class I when high-affinity peptide is bound in vivo or in vitro (Ortmann et al., 1994; Suh et al., 1994). The peptide competition and the catalysis model both show efficient peptide editing but are different from each other in four important features. First, the peptide competition model predicts a significant increase in the ADT from about 30 to about 60 min, while in the catalysis model, the ADT is essentially unchanged with the introduction of tapasin. It is known from pulse-chase experiments with the T134K (lysine 134 mutated to alanine) mutant of HLA-A2, which does not bind to tapasin, that – with all other parameters constant – export to the cell surface is slowed down significantly as a consequence of tapasin interaction, which supports the peptide competition model (Lewis et al., 1996). Second, the analysis of the abundances of the class I-
containing species in the simulation at steady-state (Table 4) shows that the peptide competition model predicts a large accumulation of peptide-receptive class I complexed to tapasin in the ER (the TC species) that corresponds to about 75% of total intracellular class I. The catalysis model, in contrast, predicts a much smaller accumulation of TC which only makes up about 8% of intracellular class I. Since most class I molecules are bound to tapasin and thus to the peptide loading complex in the ER (Peaper and Cresswell, 2008), the experimental data again support the peptide competition model. Third, in the catalysis model, the affinity of the class I molecule for the peptide is not altered in the presence of tapasin, while the peptide competition model predicts an increase in the dissociation constant. Even though kinetic measurements inside cells are difficult, an intriguing report suggests that dissociation of the same peptide from H-2K\(^{\alpha}\) is significantly faster when the class I molecule is retained inside the cell, i.e., in a complex with tapasin (Sijts and Pamer, 1997). In addition, two independent experimental approaches have reported increased dissociation of peptide from class I complexed in vitro with recombinant tapasin (Chen and Bouvier, 2007; Wearsch and Cresswell, 2007). Fourth, the intracellular retention of class I molecules that we observe when tapasin is transiently associated with the intermediate of the peptide binding reaction. Instead, it suggests that tapasin binds to the primary intracellular form, which is peptide-receptive, and causes its retention inside the cell. This agrees with the predictions of the peptide competition model.

Taken together, our simulation data, combined with experimental evidence from us and other laboratories, strongly support a scenario in which tapasin competes with the peptide for binding to class I. While other hypotheses that are being considered in the literature can explain the peptide editing effect to some extent, they lead to contradictions with experimental observations such as the release of class I from tapasin upon peptide binding, and the kinetics of transport to the cell surface. Our work thus shows that numerical model-based analysis is useful to discriminate biological hypotheses.

How could a competition between tapasin and a peptide for class I binding occur in molecular terms? Even though it is formally possible that a part of tapasin directly occupies the peptide binding groove and displaces the peptide, we believe that this is unlikely to be the case, since the binding grooves of class I molecules differ between alleles. Instead, we and others have suggested that tapasin might disturb the hydrogen bonds that tether the C terminus of the peptide to the conserved residues Lys-146 and Trp-147 of the α\(_2\)-1 helix by manipulating the conformation of that helix, pulling it outward to disrupt the hydrogen bonds, and universally increasing the dissociation rate of all class I-bound peptides.

In addition to the peptide competition model, some aspects of the catalysis model warrant further consideration. Recent molecular dynamics simulations have suggested that in the absence of peptide, the tapasin-dependent class I molecule, HLA-B*4402, exhibits a wide-open conformation of the α\(_2\)-1 helix (in the vicinity of the carboxy terminus of the peptide) in which the hydrogen bonds between the peptide C terminus and class I cannot be formed. In contrast, the tapasin-independent B*4405 shows a much more restrained α\(_2\)-1 helix in a conformation that is suitable for peptide binding (Sieker et al., 2007). If tapasin simply stabilized a wide-open conformation of class I, then peptide binding to B*4402 would be even less likely in its presence. Perhaps, therefore, tapasin binds to a conformationally more restrained peptide-free form of class I that is closer on the reaction coordinate to the peptide-bound conformation. Such an intermediate peptide-receptive form has been suggested based on peptide binding experiments with a class I-tapasin complex (Chen and Bouvier, 2007). This scenario would formally satisfy the peptide competition hypothesis, but may include elements of catalysis, i.e., an acceleration of the rate of peptide association. Since the crystal structure of tapasin has now been obtained (Dong et al., 2009), its mechanism of action can now be addressed by in silico docking simulations.

The peptide competition model makes several predictions that can be tested experimentally. First, the rate of peptide dissociation should be accelerated in the presence of tapasin. This effect has indeed been observed in two recent studies (Chen and Bouvier, 2007; Wearsch and Cresswell, 2007), but only for some peptides, and it remains to be demonstrated on a broader range of class I molecules and peptides. Such rate measurements require either an in vitro system, in which class I and tapasin interact, or a system that recapitulates class I-peptide binding in microsomal membranes, in the natural environment of the peptide loading complex (Levy et al., 1991). Second, if tapasin indeed binds preferentially to a peptide-receptive form of class I as proposed above, then in the absence of tapasin, tapasin-independent class I molecules should bind peptides faster than closely related tapasin-dependent ones. While molecular dynamics simulations support this hypothesis (Sieker et al., 2007), it remains to be tested in the experiment by association rate measurements (Springer et al., 1998). Third, many degrees of tapasin dependence and maturation efficiency between class I alleles that exist may originate from different k\(_c\), k\(_{D,e}^{-}\) or k\(_{D,p}^{-}\) values, as well as allele-specific affinities for tapasin. Substantial further experimentation will be required to clarify these points.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.molimm.2009.02.032.

References


