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

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ABSTRACT

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.

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 (β_2m), 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

Abbreviations: ADT, average dwelling time; β_2m, beta-2 microglobulin; CTL, cytotoxic T lymphocyte; ER, endoplasmic reticulum; GFP, Green Fluorescent Protein; k, reaction rate constant; K_D, equilibrium dissociation constant; MHC, major histocompatibility complex; ODE, ordinary differential equation; PLC, peptide loading complex; TAP, transporter associated with antigen presentation; TCR, T cell receptor.

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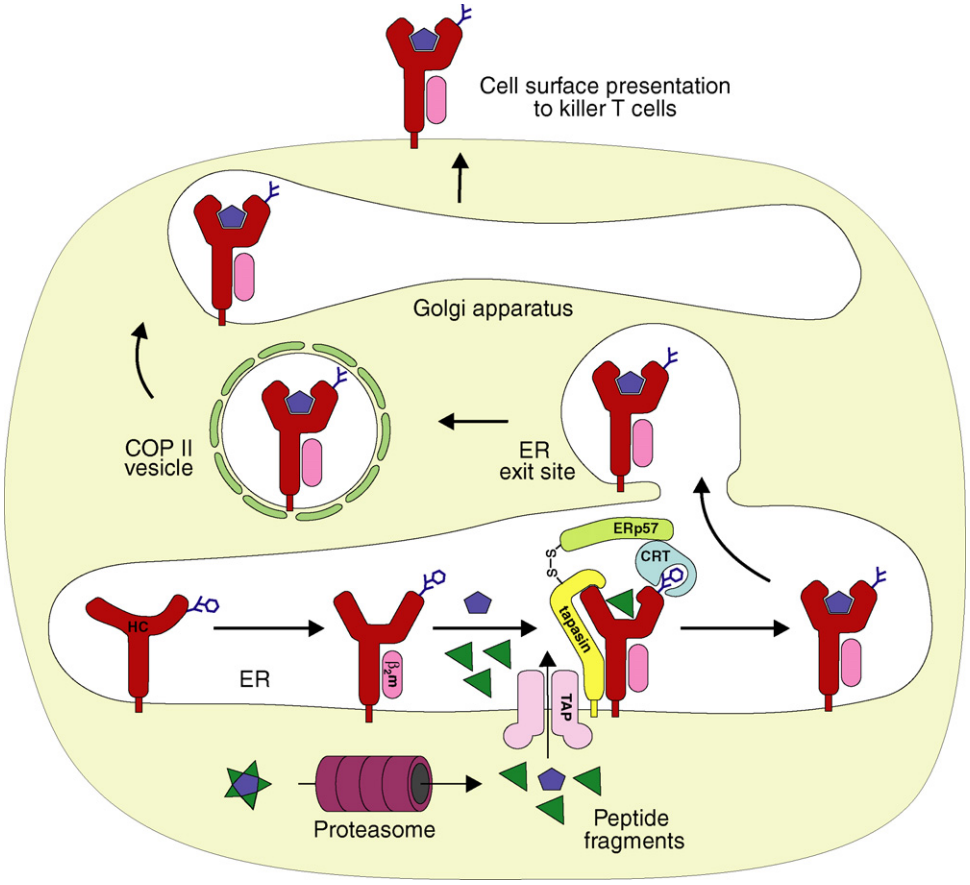

Fig. 1. MHC class I mediated antigen presentation.
MHC class I molecules assemble in the ER from the heavy chain (HC) and beta-2 microglobulin (β_2m) and bind peptides that are generated in the cytosol by the proteasome and transported into the ER lumen by the TAP transporter. Peptide binding is assisted 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-Brewer et al., 1996; Turnquist et al., 2002). Indeed, tapasin binds close to the peptide binding groove, next to the small α_2-1 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