Protein Plasticity and Peptide Editing in the MHC I Antigen Processing Pathway

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Cytotoxic T lymphocytes (CTL) of the vertebrate immune system can eliminate pathogen-infected or malignantly transformed cells offering protection from infectious and non-infectious diseases. This protection is afforded by receptors expressed on the cell surface of CTLs, with each T cell receptor recognising a specific complex of an MHC I molecule bound to a particular peptide. In humans six MHC I allotypes can be expressed, each of which may bind a variety of peptides. Thus, thousands of different pMHC complexes are present on antigen presenting cells, where they are scanned by CTL, which may be activated if a cognate ligand is encountered.

The selection of peptides for presentation at the cell surface occurs in the endoplasmic reticulum (e.r.) and proximal Golgi apparatus and is co-ordinated by cofactors: the peptide-loading complex in the e.r. (PLC- comprising the peptide transporter associated with antigen presentation TAP; the MHC-I specific chaperone tapasin; the oxidoreductase ERp57; and the lectin-like chaperone calreticulin), endoplasmic reticulum aminopeptidases (ERAP1 and ERAP2), and acting downstream of the PLC, a complex comprising the tapasin-like protein, TAPBPR (for TAP-binding protein related) and UDP-Glucosyl glucose transferase (UGGT). The biochemical function undertaken by these molecules is remarkable: to guarantee that soon after MHC I molecules are synthesised they select a repertoire of peptides that have high enough affinities to persist at the cell surface long enough to activate a T cell response. This may mean selecting low copy number peptides rapidly (in minutes) under non-equilibrium conditions.

The mechanisms underlying peptide selection are beginning to be revealed, thanks to a great deal of functional data relating to the role of cofactors that have been accumulated from site-directed mutagenesis studies to map interaction sites; and genetic ablation studies to infer functions of individual components. In essence, these have shown that:

1. All MHC I alleles can optimise their repertoire, but vary in their efficiency.

2. For all alleles, tapasin enhances this process, resulting in the optimal selection of peptides.

3. Peptide editing most likely proceeds via an iterative cycle involving peptide association and dissociation (peptide exchange), which tapasin catalyses.

4. TAPBPR can also catalyse peptide editing although it cannot substitute for tapasin in the PLC.

The field is thus working towards understanding how the structure of MHC I and its cofactors determine the peptide editing mechanism in a way that that both illustrates the catalytic peptide exchange cycle, and explains MHC I allele-specific differences. To date, structural studies have not offered easy solutions: in particular MHC I alleles with vastly different intrinsic peptide-selector function (and hence tapasin dependency) have almost identical protein crystal structures. However, two recent papers in Science [1,2], showing the protein crystal structure of a complex between peptide-receptive MHC I and the cofactor TAPBPR bring new insight to the field (Figure 1).

Figure 1

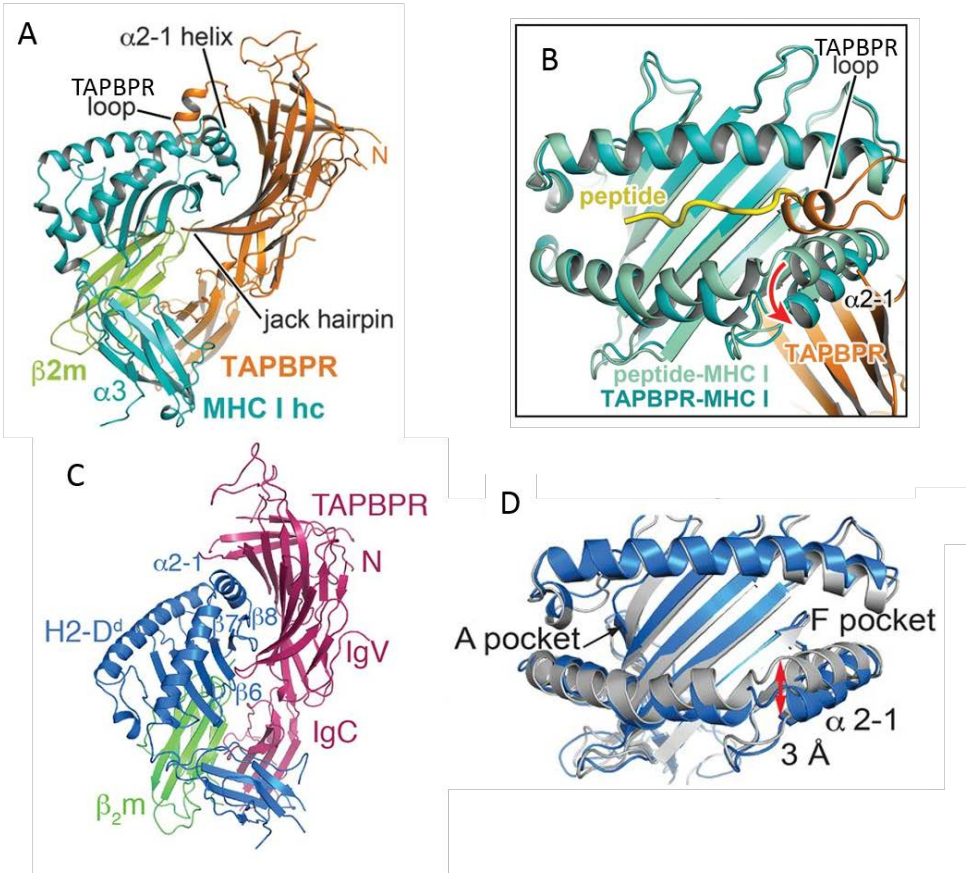

Figure 1 Structures of the TAPBPR:MHC I complex solved by Thomas and Tampe (A and B) and Jiang et al (C and D) showing the large binding interface and the hairpin loop that projects beneath the peptide binding groove of MHC I. Panels B and D show the extent of remodelling of the peptide binding groove upon binding of TAPBPR to (B) the empty MHC I and (D) MHC I artificially covalently bound to an N-terminal pentamer peptide. D shows the nomenclature for pockets accommodating the N-terminus of peptide (A-pocket) and C-terminus (F-pocket). Note also in (A) the presence of the short 3_{10} helix of TAPBPR binding to the F-pocket.

Tapasin [3] and the structurally related TAPBPR [4] have both been shown to catalyse peptide editing by MHC I *in vitro* where they bind preferentially to "peptide-receptive" MHC I molecules – generally assumed to be devoid of bound peptides. Structural studies of peptide-receptive MHC I have been difficult to undertake because peptides stabilize the

MHC I protein, and in their absence MHC deteriorates rapidly. These two new studies have employed different biochemical approaches to generate the peptide-receptive MHC I state to which TAPBPR can bind. Thomas and Tampe utilise UV-cleavable conditional peptide ligands to generate "empty" MHC upon photolysis while Jiang et al employ a combination of a dipeptide, assumed to bind in the MHC I F pocket, and a short N-terminal peptide fragment that is disulphide crosslinked to a non-native cysteine introduced into the MHC I peptide binding site. Both resulting structures show that a large region of the solvent accessible face of the MHC I protein (surrounding the part of the peptide binding groove that interacts with the C-terminal residues of the peptide ligand) is cradled by a concave surface of TAPBPR; and that a β hairpin loop of TAPBPR protrudes beneath the peptide binding domain to interact with several β strands forming the floor of the peptide binding groove as well as β-2m (Figure 1 A and C). A comparison with the native structure of MHC I (in its TAPBPR unbound) state shows that the TAPBPR-bound peptide-receptive MHC I has a more open conformation in which the α helices are displaced outwards and downwards, most notably for the α2-1 sub-helix which interacts directly with TAPBPR; and several β strands situated nearby are displaced downwards by the interaction with TAPBPR (Figure 1 B and D). Interestingly there was also a change in the relative positioning of both the membrane proximal α3 domain and β-2m.

These observations support the hypothesis that MHC I protein plasticity plays a key role in optimal peptide selection and that peptide editing is achieved by MHC I cycling between multiple conformations involving both the peptide binding domain and the α3 domain and β-2m. These define peptide–receptive or "open" conformations and native or "closed" conformations which interconvert during peptide exchange; with MHC I:peptide adducts exiting the cycle only when they have achieved a critical level of stability by incorporating high affinity peptide cargo (Figure 2).

Figure 2

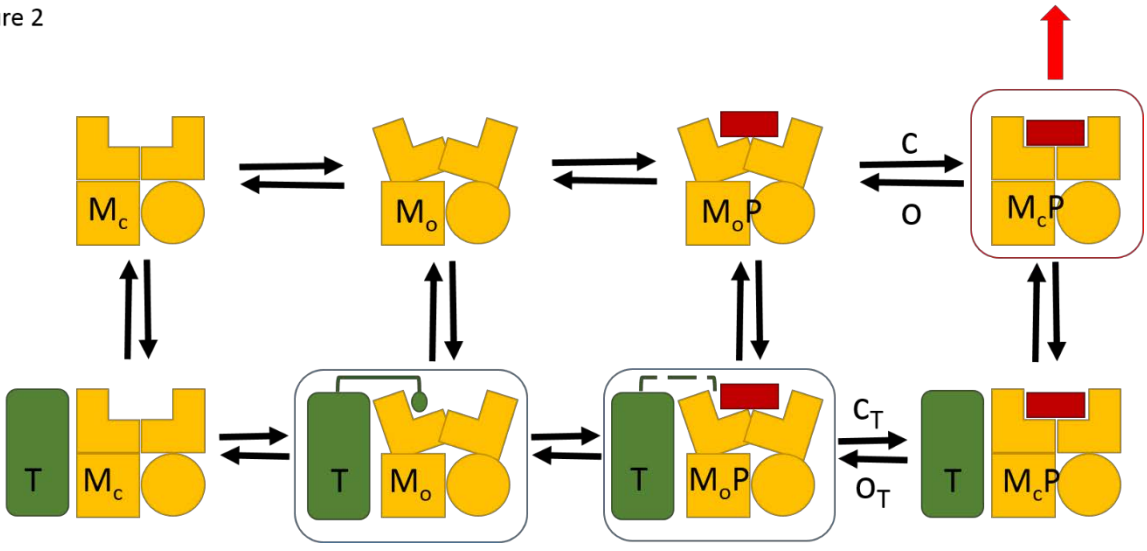

Figure 2 Schematic diagram showing the best-fit model to describe MHC I allele-dependent differences in tapasin/TAPBPR dependency for peptide cargo editing (adapted from ref 5). MHC I can exist in an open peptide-receptive conformation (Mo) or closed native conformation (Mc) and either be bound to tapasin/TAPBPR (bottom row) or not (top

row). The MHC I allele dependent parameter is “c” and the peptide dependent parameter is “o”. Binding to tapasin/TAPBPR is likely to facilitate the transition between conformations (in particular cT > c) and increase the flux through the McP complex thereby increasing the rate of peptide sampling and hence exchange, when peptides are abundant. When peptides are scarce, peptide dissociation may be the dominant outcome of tapasin/TAPBPR binding and this may be exaggerated in the case of TAPBPR by the presence of the short α helix “diving” into the peptide binding groove. McP complexes exit the exchange cycle at the point circled in red when the selected peptide cargo is of sufficient stability to make export from the e.r. (red arrow) more likely to occur than MHC I opening (o) in a particular timeframe. The diagram shows the two likely intermediate states (circled in blue) captured by the crystal structures of Thomas and Tampe (left blue panel depicts the TAPBPR-bound open, peptide-receptive MHC I complex shown in panels A and B in figure 1) and Jiang et al (right blue panel depicts the TAPBPR-bound open, peptide-occupied MHC I complex shown in panels C and D in figure 1).

The idea that the MHC I peptide binding groove might exist in an “open” peptide-receptive and “closed” peptide-bound state was first posited in the late 90’s to explain how MHC I might “trap” peptides following a peptide-induced conformational change thereby reconciling the apparent paradox of low specificity and high affinity (the typical MHC I binds many thousands of different peptides, yet these diverse complexes persist at the cell surface for many hours). More recently, computational systems model fitting [5] has shown that two-state models for MHC I peptide selection (in which MHC I molecules adopt two different conformational states) fit data better than one-state models; and moreover that the parameter that differentiates tapasin-dependent from less tapasin-dependent MHC I alleles is the rate of conversion between these two states (parameter “c” in figure 2). Tapasin independent alleles (having good intrinsic peptide selector function) have a fast conversion rate, while tapasin dependent alleles (with poor intrinsic selector function) convert more slowly. It follows from this model that tapasin/TAPBPR function could therefore be to accelerate the rate of conversion between “open” and “closed” intermediate states of MHC I (C_T > C in figure 2).

Thanks to the two new crystal structures, as well as a wealth of molecular dynamics simulations of peptide-free MHC I, we can now begin to interpret this model mechanistically. Taken together, these data are consistent with the concept that tapasin/TAPBPR stabilises an ensemble of structural intermediates, thereby lowering the energy barriers between the open and closed intermediates in their peptide-free and peptide-bound states thus facilitating their interconversion (see Figure 2). It is compelling to note that the two structures may actually represent two different intermediates among this functionally relevant ensemble: the peptide-free, open form in the Thomas and Tampe structure (panels A and B in figure 1, and TMo in figure 2) and the peptide-bound, open form in the Jiang et al structure (panels C and D in figure 1, and TMoP in figure 2) although in the latter, the peptide is not resolved. As the authors note this is “consistent with the view that the covalently bound 5mer is mobile in the complex and is no longer tethered to

the binding groove". While the absence of discernible electron density in the A pocket of the crystal suggests that the pentamer peptide is not stably ligated into the A pocket, it is entirely consistent with the possibility that the covalently bound peptide fragment occupies the N-terminal end of the peptide binding groove in multiple conformations. In the peptide-free structure, Thomas and Tampe note TAPBPR has "a short α helix that dives into the peptide binding groove" of MHC I to occupy space that is normally taken up by the two C-terminal amino acids of bound peptide. In the peptide-bound structure of Jiang et al this loop is not resolved and it is tempting to speculate that these observations are linked, and that the transient occupancy of the A pocket by the covalently tethered pentamer is allosterically communicated to the F pocket – destabilizing the interaction with the TAPBPR short α helix upon ligation with peptides that have docked at their N-terminus, and that TMo and TMoP are distinguished by their interactions in this region.

Tapasin/TAPBPR facilitated interconversion between MHC I:peptide intermediates is important for peptide exchange because computational model fitting indicates the rate of transition from closed to open state for peptide-bound MHC is peptide-dependent (parameters "o" and "oT" in Figure 2). In other words, this is the step at which peptides are sampled for their stability [5]. Thus according to this model, tapasin/TAPBPR increase the flux through the peptide-bound, closed state McP thereby accelerating the rate of peptide exchange. Precisely which contacts between peptide and MHC dominate this sampling step remains to be seen, but the current structures may give us a clue as the authors of both papers suggest engagement of Y84 could be crucial. This conserved residue lies in the F-pocket and interacts with the C-terminal carboxyl group of bound peptide in the native peptide MHC I structure but is displaced and interacts with TAPBPR in the peptide-receptive and TAPBPR complexed state. Taken together, these speculations – inspired by the structures – describe a mechanism for the co-ordinated sampling of peptide binding at both the N and C termini.

Despite their structural similarity, and similar function measured *in vitro*, there are significant differences between tapasin and TAPBPR function *in vivo*. Here, their functions are non-redundant and TAPBPR acts downstream of peptide editing by tapasin, in an intracellular compartment in which it is assumed peptides are scarce. It is therefore likely that accelerated peptide dissociation rather than accelerated peptide exchange is the dominant effect of TAPBPR; which is supported by experiments showing that the tapasin-assisted MHC I-bound peptide repertoire is further refined by TAPBPR [4]. Interestingly, when tapasin and TAPBPR sequences are compared (as they are in figure S6 of reference 1), it does not appear that tapasin has an equivalent short helical loop to that of TAPBPR that projects into the F-pocket of the peptide-free, open MHC I form in the Thomas and Tampe structure (the TAPBPR loop is shown in panels A and B of figure 1). It is easy to see how this loop of TAPBPR could act as a competitive inhibitor of interactions with the C-terminal end of the peptide to accelerate macroscopic off-rate and select against peptides with poor contacts at their N-terminus. In contrast, the potential absence of this loop from tapasin may allow peptides unimpeded access to the F pocket, making peptide loading and exchange more efficient and the dominant functional attribute of tapasin.

Another player in the antigen processing pathway, the aminopeptidase ERAP1, is present in the e.r. and early secretory pathway and has an expression pattern complimenting that of TAPBPR more so than tapasin. It's function is to trim the N-termini of MHC I - bound peptides, and it may be at the TAPBPR step in the peptide editing pathway is where the function of ERAP1 is most apparent, by modifying an intermediate N-terminally extended peptide-MHC complex in such a way as to permit optimal docking of the trimmed peptide N-terminus. There is ample evidence from molecular dynamic studies that the MHC I molecule is plastic and that allosteric communication between distal locations of the molecule are likely (for example [5]) and it is tempting to speculate that optimal docking of the peptide N-terminus could be allosterically communicated to the opposite end of the peptide binding groove, precipitating the displacement of the TAPBPR loop from the F pocket, leading to full peptide ligation and the dissociation of TAPBPR.

Clearly, there is still much to discover if we are to understand peptide editing at the atomistic level: a goal worth pursuing because of the potential benefits of being able to predict which peptides from a cancer or infection are likely to be selected by MHC I and therefore be good targets for vaccines; or of targeting the peptide editing cofactors with a view to manipulating antigen presentation using small molecules. Many of the answers will lie in direct observations of MHC I protein dynamics during a "working" peptide exchange cycle– particularly when complexed with tapasin or TAPBPR; and this calls for the integration of NMR and molecular dynamics simulation with cellular biochemistry.

References

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Acknowledgements

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