

Chapter 10

Structure and Function of Molecular Chaperones that Govern Immune Peptide Loading



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Abstract Major histocompatibility class I (MHC-I) molecules bind peptides derived from cellular synthesis and display them at the cell surface for recognition by receptors on T lymphocytes (TCR) or natural killer (NK) cells. Such recognition provides a crucial step in autoimmunity, identification of bacterial and viral pathogens, and anti-tumor responses. Understanding the mechanism by which such antigenic peptides in the ER are loaded and exchanged for higher affinity peptides onto MHC molecules has recently been clarified by cryo-EM and X-ray studies of the multimolecular peptide loading complex (PLC) and a unimolecular tapasin-like chaperone designated TAPBPR. Insights from these structural studies and complementary solution NMR experiments provide a basis for understanding mechanisms related to immune antigen presentation.

Keywords Immune recognition · Major histocompatibility complex · Chaperone · Tapasin · TAPBPR · Protein loading complex · Structural biology · X-ray crystallography

Abbreviations

MHC	Major histocompatibility complex
MHC-I	MHC class I
MHC-II	MHC class II
TCR	T cell receptor
NK	Natural killer
ER	Endoplasmic reticulum
PLC	Peptide loading complex

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TAP	Transporter associated with antigen processing
TAPBP or tapasin	TAP binding protein
TAPBPR	TAP binding protein, related
NMR	Nuclear magnetic resonance

Introduction

The critical function of the adaptive immune system is to recognize and eliminate cells whose physiological function has been dysregulated by infection or oncogenesis. Such recognition also demands discrimination of aberrant from normal cells. The ongoing evolutionary solution to this problem includes generation of a large repertoire of T lymphocytes bearing T cell receptors (TCR) in the thymus that are screened quantitatively for their reactivity to self. This is a selective process dependent on major histocompatibility complex (MHC)-encoded molecules that bind peptides and display them at the cell surface. TCRs that react strongly to self-peptide/MHC complexes are deleted in a process called negative selection. To avoid negative selection, cells bearing TCR must react at a quantitatively lower level with the same MHC molecules, a process of positive selection. The net outcome of such thymic education of T cells is the generation of a TCR repertoire that can discriminate between self and non-self. Once in peripheral lymphoid tissues, such T cells are positioned to recognize dysregulated or foreign peptides bound to self-MHC molecules, a process known as MHC-restricted recognition (Archbold et al. 2008; La Gruta et al. 2018).

Mature T cells fall broadly into those that recognize one of two categories of antigen presenting molecules: (1) MHC class I (MHC-I) that are loaded during folding and assembly in the endoplasmic reticulum (ER) with peptides generated in the cytoplasm by the proteasome or imprecise protein translation; or (2) MHC class II (MHC-II) that bind peptides derived from proteolysis in endosomes or lysosomes (Blum et al. 2013; Germain and Margulies 1993; Rock et al. 2016; York and Rock 1996). Each of these classes of MHC molecules then biosynthetically matures and arrives at the cell surface for recognition by T cells ($CD8^+$ for MHC-I, and $CD4^+$ for MHC-II). Although peptide loading in the MHC-I and MHC-II pathways has been the subject of intensive study for over two decades, the past few years have witnessed new insights derived from structural studies and molecular dynamics simulations of multimolecular complexes involved in peptide loading (Zacharias and Springer 2004). In addition to these studies contributing to our understanding of critical aspects of antigen presentation in cell-mediated immunity, they offer new insight into the broader areas of protein folding, chaperone function, and macromolecular assembly.

This review is primarily focused on the MHC-I loading pathway, with particular attention to the role of chaperone proteins that stabilize the MHC-I molecule in a

conformation accessible for peptide loading. This mode of stabilization facilitates peptide exchange favoring high affinity peptides. In particular, the chaperones designated tapasin (also known as TAP binding protein, TAPBP), and a tapasin homolog commonly called TAP binding protein, related (TAPBPR), are of current interest, because they have permitted detailed structural analyses of the mechanisms of peptide loading of the MHC-class I loading pathway. We will briefly compare the MHC-I pathway to some aspects of MHC-II loading pathway as well.

MHC Molecules, Peptide Binding Ligands for TCR

The MHC molecules are highly polymorphic heterodimeric cell surface molecules that sample peptides for display at the cell surface where they function as ligands for TCRs. Their extensive polymorphism (over 15,000 MHC-I and 5000 MHC-II alleles have been identified in humans (Robinson et al. 2015)) provides a reservoir of molecules that are capable of binding and presenting peptides derived from established as well as newly evolved infectious organisms. In addition, particular MHC types are frequently tightly linked to autoimmune diseases, immune responsiveness dictating virus resistance or sensitivity, and the resistance to tumor or tumor encoded neoantigens. Thus, understanding the detailed molecular steps involved in MHC biosynthesis, assembly, peptide loading, and cell surface expression leading to TCR engagement and TCR-mediated signaling is crucial to devising interventions to augment or contain the adaptive immune response. At the center of any molecular understanding of protein biochemical events is knowledge of the three-dimensional structure of the molecules involved, the biophysical basis of their interactions, and how those structures and molecular interactions dictate function. Recent years have seen dramatic advances in X-ray, cryo-EM, and NMR structure determination. In particular, there are now available hundreds of high-resolution models based on X-ray data of MHC-I and MHC-II molecules complexed with different antigenic peptides, and dozens of models of peptide/MHC/TCR complexes (Adams and Luoma 2013; Marrack et al. 2008; Margulies et al. 2008). However, all the structures, with some exceptions, recapitulate the original ones determined years ago (Bjorkman et al. 1987; Brown et al. 1993; Fremont et al. 1992; Garboczi et al. 1996). Summary illustrations of a representative MHC-I molecule, complexed with its light chain, the relatively invariant β_2 -microglobulin (β_2m) and a virus-derived antigenic peptide (together denoted pMHC) are shown in Fig. 10.1a–d, f. The larger molecular complex of pMHC bound to a cognate $\alpha\beta$ T cell receptor (TCR) is shown in Fig. 10.1e.

The crucial features of the extracellular domain of the pMHC complex, illustrated here by the molecular complex of the mouse MHC-I molecule, H2-D^d, is that the amino terminal domain unit, designated $\alpha 1\alpha 2$, about 180 amino acids in length, forms a peptide binding site consisting of two α -helices supported by eight strands of anti-parallel β -sheet (Fig. 10.1a). This binding site is supported by the light chain, β_2 -microglobulin (β_2m), that provides stability (Fig. 10.1b). The peptide is

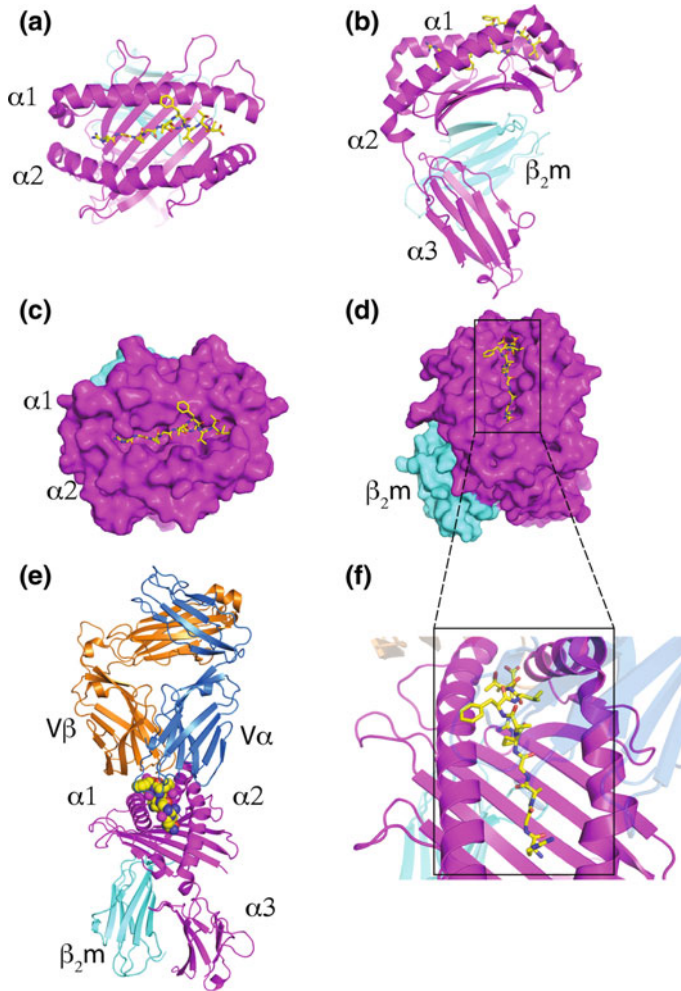


Fig. 10.1 X-ray structure of typical pMHC-I and pMHC/TCR complexes. Ribbon (**a**, **b**, **f**) and space-filling (**c**, **d**) illustrations of the crystal structure of the mouse MHC-I molecule, H2-D^d complexed with an HIV gp120-derived viral peptide, P18-I10 (RGPGRAFVTI), and β_2m are shown (resolution, 1.7 Å, PDB, 3ECB (Wang et al. 2009)). The complex of the same pMHC with a peptide-specific, MHC-restricted TCR is also shown (2.1 Å, PDB, 5IVX (Natarajan et al. 2017))

bound in a crevasse which contains six pockets (usually for MHC-I molecules designated A through F) that accommodate the amino and carboxyl termini of the peptide as well as several side chains of the peptide (Fig. 10.1c, d, f) (Saper et al. 1991). In general, the most important pockets are at the amino and carboxy termini of the peptide (the A, B and F pockets respectively). The peptide is displayed distal to the cell's plasma membrane, and the complex of the peptide bound to the MHC-I molecule provides a molecular surface available for interaction with the variable (V domains) of the TCR α and β chains (Fig. 10.1e).

Peptide Loading in the MHC-I Pathway

Early studies recognized that cells deficient in peptide transport into the ER (i.e. defective in expression of the heterodimeric ATP-dependent transporter associated with antigen processing, TAP) expressed low levels of surface MHC-I, a phenotype partially reversed by culturing the mutant cells at room temperature rather than at 37 °C (Ljunggren et al. 1990; Spies and DeMars 1991). Defects in the transport of peptides from the cytoplasm to the ER provided a deficiency in available peptides, resulting in fewer available high affinity peptides, and thus a lower steady state concentration of thermally stable cell surface MHC-I molecules. Several decades of biochemical studies lead to the general model illustrated in Fig. 10.2. Peptides generated in the cytoplasm by defective translation or by proteolytic digestion of larger proteins by the proteasome are released to be transported by the TAP1/2 transporter of the PLC into the ER (Fig. 10.2a). There, the peptides may be bound by the peptide-receptive MHC molecule of the PLC, can be trimmed at the amino-terminus by the ERAP endopeptidases (Chen et al. 2016; Serwold et al. 2002), and are submitted to high affinity quality control mediated by the tapasin chaperone. Once a peptide of sufficient affinity is bound, the pMHC-I/ β_2m complex proceeds through the Golgi to the cell surface. The glycosylation status of the folded MHC-I serves as an additional quality-control step, mediated by the interaction with UDP-glucose glycoprotein glucosyltransferase (UGT1) (Zhang et al. 2011).

Structure of Tapasin/ERp57

Understanding the role of tapasin as a component of the PLC has been elucidated by several experimental approaches: genetic studies evaluating the function of cells and animals defective in tapasin expression (Copeman et al. 1998; Garbi et al. 2003; Grandea et al. 2000), biochemical studies exploring the role of tapasin in facilitating peptide loading (Sadasivan et al. 1996; Chen and Bouvier 2007; Wearsch and Cresswell 2007), and structural studies of tapasin (Dong et al. 2009). A major step in understanding the structural contribution of tapasin to peptide loading was achieved by the determination of the X-ray crystallographic structure of a molecular complex of tapasin with ERp57, the oxidoreductase component of the PLC (Dong et al. 2009). By coexpressing the luminal domains of both tapasin and ERp57 as a disulfide-linked unit, the authors successfully produced heterodimers that crystallized, and then determined the X-ray structure at 2.6 Å resolution (Fig. 10.3). Two heterodimers of tapasin/ERp57 are contained in the asymmetric unit, with several

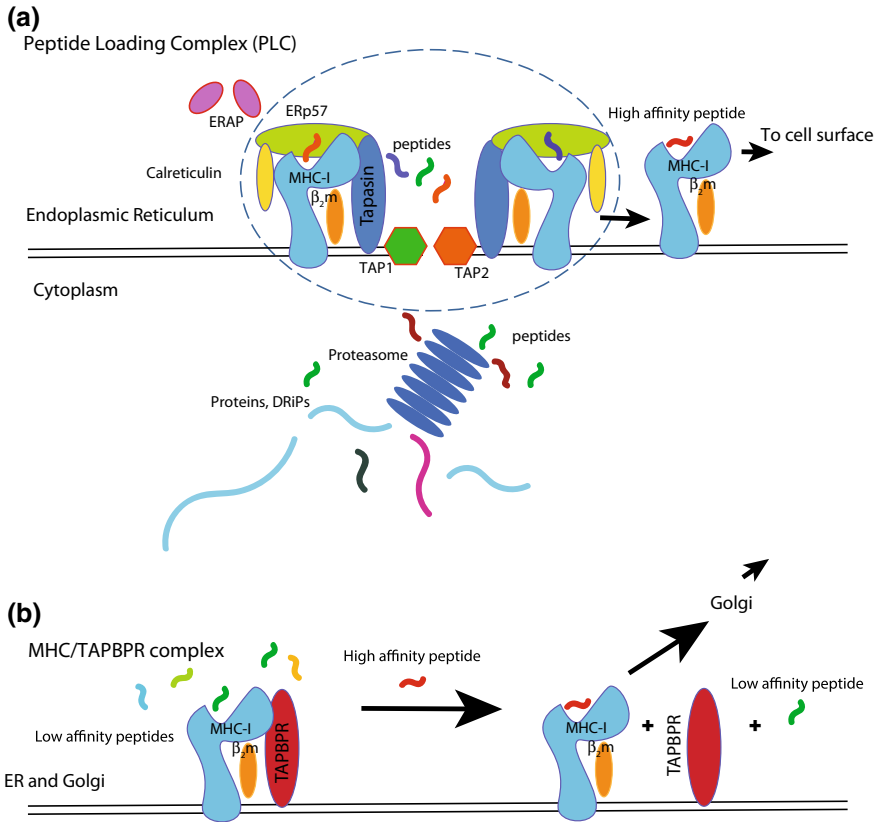


Fig. 10.2 Peptide loading by the PLC or by the PLC-independent TAPBPR. **a** Schematic representation of the key molecules involved in peptide loading. Cytoplasmic proteins or defective ribosomal products (DRiPs (Yewdell and Nicchitta 2006)) are further degraded by the proteasome to a length of about 8–15 amino acids, transported to the ER via the TAP1/2 ATP-dependent transporter, where under the influence of the full PLC they are loaded on MHC-I molecules. Amino terminal trimming by ERAP occurs during this process. MHC-I complexed with high affinity peptides proceed to the Golgi and to the cell surface. **b** the MHC/TAPBPR complex functions independent of the PLC and allows exchange for high affinity peptides. Not shown are additional quality control steps based on interaction with UGT1 (Zhang et al. 2011; Neerinx et al. 2017)

loops of tapasin in regions of poor electron density indicative of disorder in the crystal, and consistent with mobility in solution. Of major interest is the structure of the tapasin component itself, and the structural location of amino acid residues that affect MHC-I interaction. The interacting amino acid residues were established by site-directed mutagenesis and the resulting impairment of peptide loading, the key PLC function. Using a cell-free system (Wearsch and Cresswell 2007), a number of tapasin mutants were identified that defined a conserved surface of the tapasin molecule (Fig. 10.3b). These mutants not only severely impeded PLC function, but

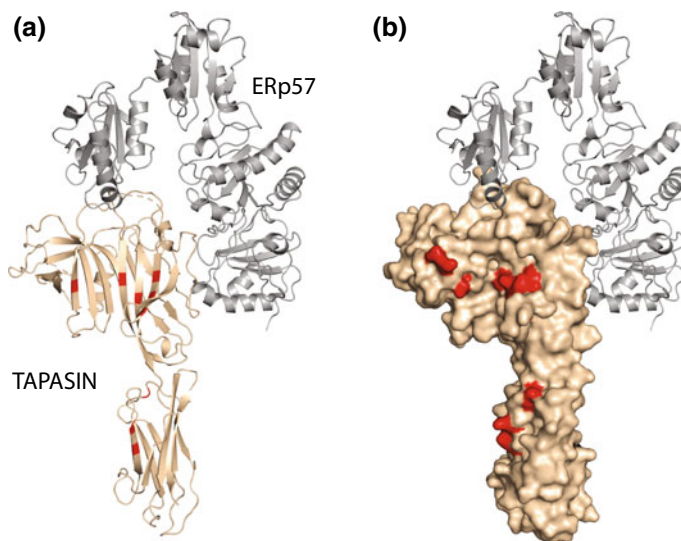


Fig. 10.3 Structure of tapasin/ERp57 reveals location of residues that affect function in MHC-I peptide loading. **a** Ribbon diagram of the tapasin/ERp57 heterodimer (2.6 Å, PDB, 3F8U (Dong et al. 2009)), tapasin, magenta; ERp57, grey. Residues that impaired peptide loading capacity are indicated in red. **b** Surface representation of tapasin, nestled into U-like clasp of ERp57. Same color scheme as (a)

also impaired their ability to bind MHC-I molecules. Most strikingly, one mutant also failed to restore cell surface expression of the highly tapasin-dependent HLA-B*44:02 molecule in a tapasin deficient cell line. The structural analysis of the tapasin/ERp57 complex, studies of monoclonal antibody reactivity, and molecular modeling and docking strategies formed the basis of several working models of the structure of the entire PLC (Dong et al. 2009; Koch and Tampe 2006; Panter et al. 2012; van Hateren et al. 2013; Van Hateren et al. 2010). Since the PLC contains not only MHC-I, tapasin and ERp57, but also the lectin-like chaperone calreticulin and the heterodimer TAP, understanding the structural relationship of the MHC-I to tapasin remained a critical point in developing a mechanistic view of the function of the PLC. High resolution appreciation of the interaction of tapasin with MHC-I continues to be an elusive undertaking.

Low Resolution Structures of Tapasin and TAPBPR

When the front door is locked, it is often helpful to see if a side door is open. Difficulties in assessing structural aspects of PLC function and in particular the role played by tapasin in chaperoning peptide-receptive MHC-I have been overcome in part by studies of the tapasin-like molecule, TAP binding protein, related

(TAPBPR). First identified as a gene encoding a molecule with low ($\sim 22\%$) amino acid sequence identity to tapasin, the TAPBPR protein was initially characterized as a member of the immunoglobulin superfamily. Biochemical studies found TAPBPR predominantly in the endoplasmic reticulum (Teng et al. 2002). Subsequently, Boyle and colleagues explored the molecules associated with TAPBPR, and found that, in contrast to tapasin, it is associated with MHC-I and not with other components of the PLC (Boyle et al. 2013). Also, during biosynthesis and maturation TAPBPR remains in complex with MHC-I past the ER and through the Golgi. Further studies demonstrated the ability of TAPBPR to interact strongly with MHC-I molecules devoid of peptide (Hermann et al. 2015; Morozov et al. 2016), with differential selectivity for varied MHC-I allelic protein products. Mutational analyses of TAPBPR, based on amino acid sequence similarity with tapasin (Hermann et al. 2013; Morozov et al. 2016) supported the view that TAPBPR functioned as a tapasin homolog, and exploited a similar MHC-I binding site. Using human HLA-A*02:01 emptied of bound peptide by photolysis of a UV-sensitive peptide, analysis by size exclusion chromatography, analytical ultracentrifugation, and surface plasmon resonance established the ability of TAPBPR to bind peptide-free MHC-I molecules. TAPBPR interacts with a wide variety of MHC-I allelomorphs, and the human molecule binds some murine MHC-I molecules as well (Morozov et al. 2016).

Efforts to clarify the three-dimensional structure of tapasin bound to MHC-I, however, were initially stymied by the lack of suitable crystals. One alternative approach was to obtain low resolution envelope structures of tapasin and TAPBPR, using small angle X-ray diffraction. The experimentally based models revealed similar solution structure of the two molecules in their luminal domains (Morozov et al. 2016). The low resolution structure of TAPBPR provided encouragement for the biochemical purification of crystallizable TAPBPR/MHC-I complexes.

X-Ray Structures of TAPBPR

Diffraction quality crystals were obtained in two laboratories, leading to X-ray based models of MHC-I/TAPBPR complexes (Jiang et al. 2017; Thomas and Tampe 2017). Jiang et al. produced the mouse MHC-I molecule, H2-D^d disulfide linked to a carboxyl terminally truncated 5-mer peptide, RGPGRC, that occupied the amino-terminal region of the peptide binding groove, leaving the carboxyl-terminal part stabilized by a glycine-leucine dipeptide (as suggested by refolding studies of Saini et al. 2013). Using surface plasmon resonance, they demonstrated that this molecule, lacking the usual carboxyl terminal anchor in the F pocket, bound rather tightly to human TAPBPR (hTAPBPR). X-ray data sets for crystals of the peptide 5-mer/H2-D^d complex (to 2.7 Å resolution, PDB, 5WES) as well as for the 5-mer/H2-D^d/TAPBPR complex (to 3.4 Å, PDB, 5WER), generated refined models for analyzing the contacts between TAPBPR and the H2-D^d MHC-I molecule as well as the conformational differences distinguishing the bound and

unbound MHC-I molecule. Figure 10.4a illustrates the general features of the MHC-I/TAPBPR complex (Fig. 10.4a), the strong structural similarity of TAPBPR to tapasin (Fig. 10.4b), and the conformational changes observed in the MHC-I molecule before (Fig. 10.4d) and after (Fig. 10.4a, c) interaction with the TAPBPR chaperone. Detailed comparisons, based on superposition of the unbound and

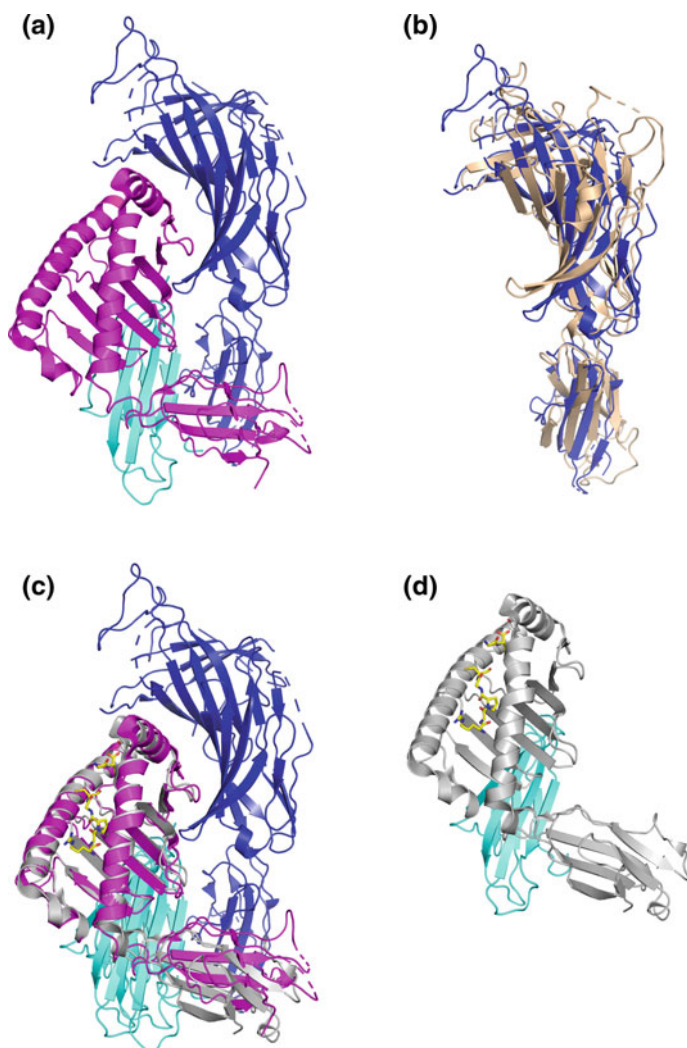


Fig. 10.4 Structure of the complex of TAPBPR with the H2-D^d MHC-I molecule. **a** Complex of H2-D^d/β₂m/TAPBPR complex (3.4 Å, PDB, 5WER), H2-D^d, magenta; β₂m, cyan; TAPBPR, blue. **b** superposition of TAPBPR onto tapasin, tan (PDB, 3f8U). **c** Superposition of the 5-mer peptide/H2-D^d(grey)/β₂m complex (2.7 Å, PDB, 5WES) onto the H2-D^d/β₂m/TAPBPR complex (5WER). **d** Unliganded, 5-mer peptide/H2-D^d/β₂m, for comparison

bound MHC-I molecule (Fig. 10.5) revealed a number of striking details (Fig. 10.5). The α 2-1 helix of the MHC molecule is displaced by as much as 3 Å on interaction with TAPBPR. A key amino acid residue of the MHC, Tyr84 is repositioned by interaction with TAPBPR Glu102, drawing it away from its usual position anchoring the carboxylate group of the C-terminal amino acid of the bound peptide (Fig. 10.5a, c). The floor of the peptide binding groove is also displaced by about 1.8 Å (Fig. 10.5b), apparently related to interactions with a Lys211 to Arg213 loop of TAPBPR. The interaction of the membrane proximal Ig-like domain of TAPBPR with both the membrane proximal α 3 domain of the MHC-I

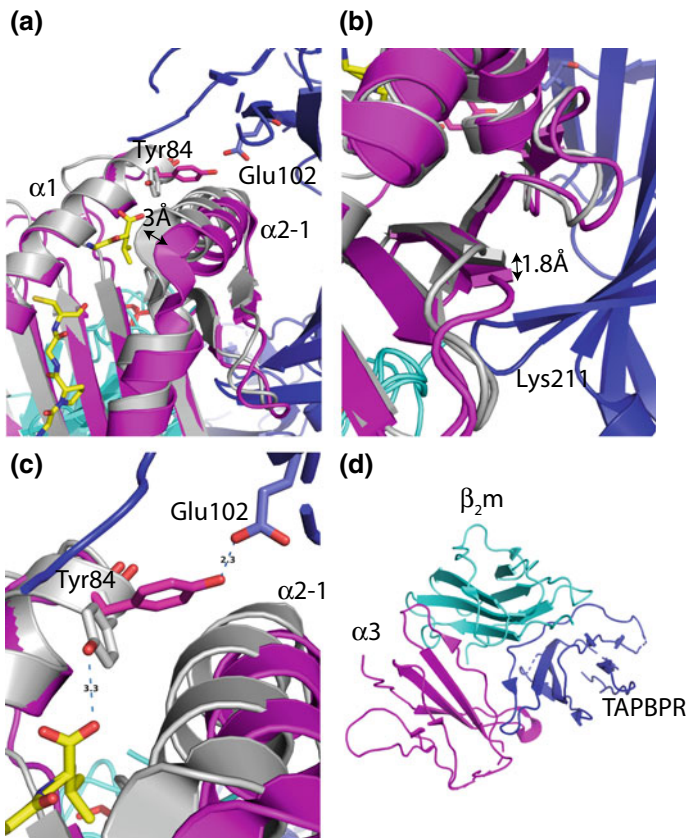


Fig. 10.5 Conformational changes of pMHC accompany TAPBPR interaction. **a** Close-up view of the peptide-binding groove of the pMHC (grey) superposed on the pMHC/TAPBPR complex (α 1 and α 2, magenta), emphasizing the 3 Å displacement of the α 2-1 helix. **b** Close-up of the floor of the binding groove, showing displacement of the β 8-strand and the position of the Lys211 to Arg213 (not labelled) loop of TAPBPR. **c** Change in orientation of side chain of Tyr84 of H2-D^d from the unliganded (grey) to the TAPBPR-bound state (magenta) where it forms hydrogen bonds to Glu102 of TAPBPR. **d** Top down view of the membrane proximal Ig-like domains of H2-D^d heavy chain (magenta), β ₂m (cyan) and TAPBPR (blue)

heavy chain and with the β_2m subunit, forming a trimeric structure, not predicted by any of the dynamics simulations was another unanticipated finding (Fig. 10.5d). Most remarkably, although the MHC-I molecule used to provide a ligand for TAPBPR in these studies contained a truncated peptide covalently linked via a disulfide bridge to an engineered cysteine residue at position 73, clearly observed in the density map of the unliganded MHC-I molecule, the complex with TAPBPR however showed no consistent electron density for the peptide. Also, the general structure of the peptide binding groove was rearranged to a more open conformation. Importantly, the structure of MHC-I/TAPBPR shows that low affinity peptides are released when chaperone binds to MHC-I, and when a high affinity peptide is bound the chaperone is disassociated from MHC-I. The mechanism of “peptide editing” in the absence of the PLC is shown in Fig. 10.2b.

The studies of Jiang et al. (2017) are remarkably consistent with the structure determination of Thomas and Tampé (Thomas and Tampe 2017) who used a slightly different strategy to produce H2-D^b/TAPBPR complexes and crystals. They expressed and purified the mouse MHC-I molecule H2-D^b with a photolabile viral peptide. Following UV photolysis, the H2-D^b/hTAPBPR complex produced crystals that diffracted to 3.3 Å (PDB, 5OPI). The structural models produced by the two groups are very similar. Superposition of 5OPI (containing one MHC/TAPBPR complex) on 5WER (which has four complexes per asymmetric unit) gives an RMSD of 1.163 Å. The individual complexes of 5WER range in RMSD from 0.934 to 1.467 Å. However, the 5OPI structure was modeled with an α helix for residues 27–34, a region that produced no electron density in the 5WER data sets. Indeed, the lack of density in the 5WER refined data, and large crystallographic *B*-factors for these residues in the 5OPI structure (averaging about 247 Å² for backbone atoms, consistent with a thermal motion (*u*) of such atoms of greater than 1.6 Å) suggest that this is a region of high mobility, and of uncertain structure.

Functional and Structural Examination of the 22 to 35 Loop Region of TAPBPR and Tapasin

The potential role of the loop region encompassing amino acids 22 to 35 of TAPBPR and of the shorter (10 amino acid long) homologous region of tapasin in direct interaction with the peptide-binding groove of MHC-I has been studied in functional studies (for the TAPBPR loop) by Ilca et al. (2018) and for the shorter tapasin loop by X-ray studies (Hafstrand et al. 2019). Deletion and mutagenesis of the TAPBPR loop support its importance in mediating efficient peptide exchange, and focus on the role of a central leucine residue (L30) (Ilca et al. 2018). Structural studies examined a complex between a decamer peptide representative of the tapasin loop bound to the mouse MHC-I molecule H2-D^b containing a truncated viral peptide and compared this to H2-D^b and H2-K^b with the Gly-Leu dipeptide in the C-terminal F pocket (Hafstrand et al. 2019). Although the tapasin derived decamer

loop peptide was not fully visualized, all three structures indicated that the leucine side chain was capable of interacting tightly with the F pocket. Taken together, the functional studies and the structural studies are consistent with a role of the 22 to 35 loop of TAPBPR and the shorter tapasin loop in providing some steric effects affecting access of other peptides to the chaperone-stabilized binding groove. Indeed, the lack of definitive electron density for the TAPBPR loop in the MHC-I/TAPBPR structures suggests thermal mobility of the loop when the chaperone is bound to MHC-I.

Cryo-EM Structure of the PLC

Recent improvements in cryo-electron microscopy provided an opportunity to gather a structural view of the entire PLC. Using a TAP-binding herpes virus-encoded protein, ICP47, to pull down the entire PLC, Brees and colleagues isolated detergent-solubilized cross-linked complexes from Burkitt's lymphoma cells (Brees et al. 2017). These complexes consisted of TAP1, TAP2, ERp57, calreticulin, MHC-I heavy chains (likely HLA-A and -B as well as -C) and the MHC-I light chain β_2m (Brees et al. 2017). Though somewhat biochemically heterogeneous, the cryo-EM images provided pictures of the macromolecular PLC complex measuring $150 \text{ \AA} \times 150 \text{ \AA}$ across and a full 240 \AA through the membrane (see Fig. 10.6). The ER luminal domains have two editing (i.e. MHC-I containing) modules, consistent with earlier biochemical studies (Panter et al. 2012), and the heterogeneity of the structures suggested considerable dynamic flexibility. The dimeric modules were able to be refined to about 7.2 \AA resolution, while careful selection of single modules improved the resolution to 5.8 \AA . Looking down on the PLC from the top (Fig. 10.6c, d), one sees the central organization of the complex based on the dimerization of tapasin, centered on the transmembrane TAP1/2 heterodimer, with the other components radiating from this central region. Since high resolution X-ray structures of virtually all the components have been determined (with the exception of the putative long α -helix of calreticulin), a representative high resolution model of the complex was fitted into the lower resolution cryo-EM map (Fig. 10.6b, d, and f). Tapasin, itself bracketed by ERp57, supports the MHC-I, which is engaged by the calreticulin lectin domain, which then extend a long loop towards ERp57. Thus, one can envision TAP delivering peptides from the cytoplasm to the luminal side of the complex, with rapid access to the tapasin-chaperoned, peptide-receptive, MHC-I binding cleft. Since several different states of assembly were identified in the cryo-EM pictures, some indications of the dynamics of the functional complex are intimated.

Assessing the Dynamics of MHC/TAPBPR Interaction with NMR

With X-ray structures of MHC-I and MHC-I/TAPBPR complexes at hand, it is now possible to couple these data with nuclear magnetic resonance (NMR) analysis of the interactions as they occur in solution at room temperature. To this end, McShan et al. (2018) used selective heavy isotope labelling of subsets of amino acids of the

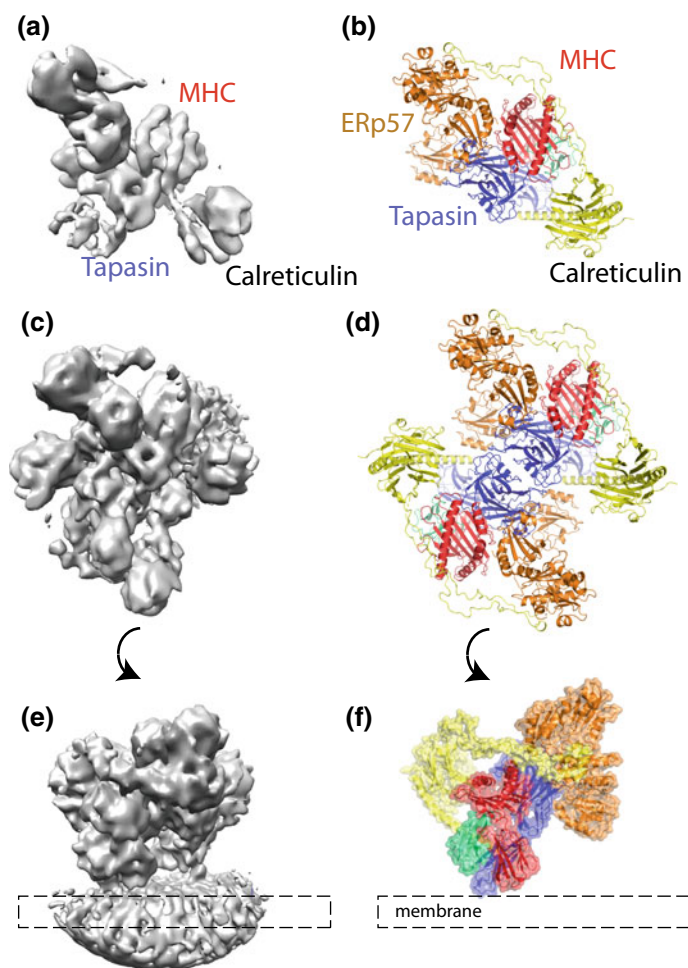


Fig. 10.6 Cryo-EM images and superposition based models reveal organization of the PLC. **a** Cryo-EM based structure taken from the electron microscope database (EMDB) (Lawson et al. 2011) entry 3905 showing the map generated by Blee et al. (Blee et al. 2017). **b** is the high resolution model deposited as pdb, 6ENY, indicating the individual proteins of the PLC. **c, e** show the map of the full complex (EMDB 3904), visualized from above (**c**) and from the side (**e**), with corresponding higher resolution images from 6ENY in (**d**) and (**f**)

MHC-I molecule, H2-D^d to experimentally address the dynamics of TAPBPR-dependent chaperoning and peptide exchange. In the peptide/H2-D^d/β₂m complexes, several regions of increased mobility including several loops and the Ig-like α3 domain were observed. Regions of MHC-I flexibility corresponded to regions of TAPBPR interaction. Analysis of Ala, Ile, Leu, and Val (AILV) methyl groups of the MHC-I revealed interaction of TAPBPR with regions completely consistent with the X-ray structures described above. Further analysis indicated that both TAPBPR and incoming peptide suppress the dynamics of the groove of the MHC-I leading to the conformational changes observed in the α2-1 helix. Most remarkably, the NMR analysis permitted detection of conformational intermediates that occurred during peptide-exchange. While peptide is binding, a peptide/MHC/TAPBPR intermediate is detected, and the final peptide/MHC-I conformation releases the TAPBPR. Contributions of interactions of peptide in the A and B pockets as well as evidence for a negative allosteric coupling between the A and F pockets is observed. Thus, NMR spectra provide solution, room temperature characterization of the dynamics of both empty and peptide-loaded MHC-I when bound to TAPBPR, and provide a model that can be extrapolated to the function of tapasin in the PLC.

Conclusions

The biological evolution of the complex cellular phenomena that are required for T cell dependent immune surveillance has been based on a remarkable series of protein/protein and peptide/protein interactions. The MHC-I loading pathway, described above, has exploited general aspects of protein folding and assembly, and the role of chaperones such as tapasin and TAPBPR to stabilize conformations to allow peptide binding. Although the details of the MHC-II loading pathway are quite different, occurring at least in part in acidic endosomal or lysosomal cellular compartments rather than in the ER, and dependent on unique chaperones, the invariant chain (Ii) and the MHC-II-like HLA-DM, the general principle that empty MHC molecules benefit from the stabilization provided by particular chaperones to facilitate peptide loading is preserved (Mellins and Stern 2014; Sadegh-Nasseri 2016; Sadegh-Nasseri et al. 2008). The ongoing improvements in our understanding of peptide loading in both the MHC-I and MHC-II pathways should be expected to not only foster deeper and broader understanding of a variety of processes in protein assembly and folding, but may provide insight into new avenues for effective immunization for infection and treatment for autoimmunity and cancer.

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