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A transmembrane tail: interaction of tapasin with TAP and the MHC class I molecule

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Abstract

The transmembrane protein tapasin has an essential role in the assembly of stable major histocompatibility (MHC) class I/peptide complexes. Within the endoplasmic reticulum, tapasin associates with both the transporter associated with antigen processing (TAP) and the MHC class I molecule. The tapasin/TAP association has been clearly shown to involve the transmembrane domains (TMD) of both molecules and to result in the stable expression of TAP. Although the influence of tapasin on MHC class I molecule folding and surface expression has been extensively studied, relatively little is known at the structural level regarding the interaction between tapasin and the MHC class I molecule. Here we summarize our current understanding of functions involving the tapasin TMD and propose that, beyond stabilizing TAP, the tapasin TMD may also interact with the MHC class I heavy chain.

Keywords

Antigen presentation/processing; Major histocompatibility complex; Tapasin; Transporter associated with antigen processing; Transmembrane

Introduction

Antigenic peptides presented by major histocompatibility complex (MHC) class I complexes alert CD8⁺ T-cells to virus infection and to tumor cells. In the endoplasmic reticulum (ER), efficient peptide loading relies on the interaction between the MHC class I molecule and a multi-protein assembly complex of which the transmembrane protein tapasin is a crucial component. Folding and assembly of MHC class I molecules has been reviewed extensively elsewhere (Garbi et al., 2007; Raghavan et al., 2008; Wearsch and Cresswell, 2008). Briefly, tapasin bridges the MHC class I heavy chain (HC)/ β_2 -microglobulin heterodimer to the

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transporter associated with antigen processing (TAP) (Sadasivan et al., 1996). TAP transports peptides generated by the proteasome from the cytosol into the ER lumen. Other components of the peptide-loading complex include the thiol oxidoreductase ERp57 and the lectin chaperone calreticulin. Here we focus on the role of the tapasin transmembrane domain [TMD (Table I)] within the loading complex.

Tapasin/TAP Interaction

TAP consists of two subunits, TAP1 and TAP2. Each subunit is composed of several membrane-spanning helices and a cytosolic domain involved in binding ATP (reviewed in Raghavan et al., 2008). Association of tapasin with TAP occurs via the tapasin TMD and the N-terminal transmembrane helices of TAP1 and TAP2 (Lehner et al., 1998; Tan et al., 2002; Koch et al., 2004; Leonhardt et al., 2005; Procko et al., 2005; Koch et al., 2006; Papadopoulos and Momburg, 2007). Through this interaction, tapasin increases the thermostability of TAP1 and TAP2, thereby enhancing TAP protein expression (Raghuraman et al., 2002). Indeed, in the absence of tapasin, TAP protein levels are reduced by approximately 3 fold in human cells and 300 fold in mouse cells (Lehner et al., 1998; Garbi et al., 2003). In this manner, tapasin increases the peptide supply within the ER.

From an extensive mutational analysis of mouse tapasin, Papadopoulos and Momburg (2007) proposed that a TAP-interaction motif consists of residues F397/F401/G405/K408/W412 within the mouse tapasin TMD. These residues are predicted to be located along the same flank of the membrane-spanning α -helix of tapasin. Independently mutating single residues within this motif did not disrupt TAP2 stabilization; however, combined mutation of 3 or 4 residues compromised TAP2 protein levels (Papadopoulos and Momburg, 2007).

A conserved lysine at position 408 (K408) in human, mouse and rat tapasin is predicted to lie within the TMD (Papadopoulos and Momburg, 2007; Ortmann et al., 1997; Deverson et al., 2001). Situation of a basic charge within the hydrophobic transmembrane region is energetically unfavorable (Hessa et al., 2005; Ulmschneider et al., 2005). However, charged residues within a TMD can be tolerated if a sufficient number of surrounding nonpolar residues are present (Hessa et al., 2005). Interestingly, mutation of K408 in human versus mouse tapasin has differing effects. In human cells, mutation of human tapasin K408 to alanine (K408A) or tryptophan (K408W) abrogated the tapasin/TAP interaction and caused failure of TAP stabilization (Petersen et al., 2005). However, in mouse fibroblasts, the mouse tapasin K408W mutation did not disrupt the tapasin/TAP association or TAP association with the MHC class I molecule, H2-K^d (Simone et al., 2009 and unpublished observation). Similarly, the mouse tapasin K408A mutation did not disrupt TAP2 stabilization (Papadopoulos and Momburg, 2007). It has been suggested that neighboring leucine residues in mouse tapasin may stabilize K408, and that the overall hydrophobicity of the tapasin TMD is important for TAP stabilization (Papadopoulos and Momburg, 2007). Since several residues in the human tapasin TMD are less hydrophobic than equivalent residues in mouse tapasin, TAP stabilization may be more readily compromised in the human (Papadopoulos and Momburg, 2007).

Tapasin/MHC Class I HC Interaction

Despite its ability to stabilize TAP, mouse tapasin K408W negatively impacted the assembly of H2-K^d molecules, as was evidenced by an increased level of open, peptide-free forms of H2-K^d and impaired surface stability of H2-K^d (Simone et al., 2009). Additionally, mouse tapasin K408W was found to interact more strongly with H2-K^d molecules and increased the retention of H2-K^d within the ER (Simone et al., 2009). Similarly, human tapasin K408A associated more strongly with HLA-B8 (Petersen et al., 2005). Several possibilities could explain the increased association between tapasin K408 mutants and the MHC class I HC. Removal of a positive charge within the tapasin TMD may alter the dynamics of the tapasin/

MHC class I HC interaction such that the MHC class I molecule is not easily displaced upon binding peptide. Alternatively, tapasin K408 mutants may poorly assist in the loading of high-affinity peptides such that fewer MHC class I HC/peptide complexes achieve the folded conformation required to be released from tapasin. As a third possibility, tapasin K408 mutants may influence the peptide pool transported by TAP such that there are fewer appropriate MHC class I ligands.

In total, these observations suggest that apart from its interaction with TAP, the tapasin TMD contributes significantly to the tapasin/MHC class I HC association. Whether the tapasin TMD interacts directly with the MHC class I HC remains to be resolved. It is plausible that the tapasin transmembrane helix ends at K408. As it has been noted previously, one might envision that the structure of the tapasin N-terminal helix is similar to that of the FD coat protein wherein the transmembrane helix is followed by a short turn, allowing the remaining hydrophobic sequence to rest on the membrane surface (Papadopoulos and Momburg, 2007; Marassi and Opella, 2003). Such a conformation would expose K408 to the cytoplasmic side of the ER membrane where it would be in position to interact with conserved arginine residues located at the cytoplasmic end of the MHC class I HC TMD (Fig 1). Repulsive forces between lysine and arginine should contribute to the destabilization of the tapasin/MHC class I HC association, thereby facilitating the release of the MHC class I HC upon binding peptide. This may explain the increase in MHC class I HC/tapasin association upon mutation of tapasin K408.

Identification of a disulfide-bonded complex between the MHC class I HC and human tapasin substantiates the potential for MHC class I molecules and tapasin to interact via the TMD and/or cytoplasmic tail (Chambers et al., 2008). Unexpectedly, Bulleid and co-workers (Chambers et al., 2008) found that a cysteine residue located within or on the border of the HLA-B35 TMD was involved in disulfide bond formation with tapasin. Although not formally demonstrated, it is likely that a cysteine residue present at the cytoplasmic end of the human tapasin TMD forms this disulfide linkage with HLA-B35 (Chambers et al., 2008) (Fig 1). Furthermore, the MHC class I HC/tapasin disulfide formed only in the presence of TAP, suggesting that orientation and/or conformational changes induced upon integration into the loading complex may permit transmembrane interactions between MHC class I molecules and tapasin (Chambers et al., 2008). At this point, it is unknown whether the MHC class I HC TMD/tapasin disulfide linkage occurs *in vivo* or only in the redox conditions of the *in vitro* experiments performed (Chambers et al., 2008). Furthermore, tapasin sequences from several other species, including mice, rats and cattle, do not contain a cysteine within the transmembrane/cytoplasmic region. Nevertheless, this finding indicates that within the loading complex the transmembrane regions of the MHC class I HC and tapasin are in close enough proximity to interact.

Truncation mutants of both the MHC class I HC and tapasin have been useful in examining transmembrane interactions within the loading complex. Soluble human MHC class I HC maintains an association with TAP, albeit at a compromised level (Carreno et al., 1995; Suh et al., 1996). Replacement of the MHC class I HC transmembrane and cytoplasmic regions with a GPI anchor improves the TAP/MHC class I HC association, implying that an interaction between the TMD of the MHC class I HC and tapasin is not essential for incorporation of MHC class I molecules into the loading complex (Suh et al., 1996). Also, association between soluble human tapasin and the MHC class I HC has only been detected with the use of a chemical cross-linker, indicating that this interaction is weak and/or transient (Lehner et al., 1998). Although soluble tapasin facilitates human MHC class I surface expression, these MHC class I molecules are less stable than those assembled in the presence of full length tapasin (Tan et al., 2002). Moreover, mouse soluble tapasin does not facilitate the folding or surface expression of mouse MHC class I molecules, indicating that mouse MHC class I molecules are more dependent on the tapasin TMD than are their human counterparts (Simone et al., 2009). This phenotype was not due to the inability of soluble tapasin to stabilize TAP, since co-expression

of soluble tapasin and a tapasin mutant ($\Delta 334\text{--}342$), which stabilizes TAP but does not bind to the MHC class I HC, failed to restore mouse MHC class I surface expression (Simone et al., 2009). Altogether, it is likely that interactions between the transmembrane regions of tapasin and the MHC class I HC improve MHC class I assembly with peptide.

Concluding Remarks

Of all of the intermolecular interactions occurring within the MHC class I loading complex, that between the MHC class I HC and tapasin is the least well characterized, perhaps owing to its transient nature. Several regions within the ER-luminal domains of the MHC class I HC and tapasin have been implicated in their association (Turnquist et al., 2002). Recently, a crystal structure including the luminal domains of tapasin has allowed the definition of tapasin residues involved in MHC class I heavy chain association (Dong et al., 2009). However, the potential for transmembrane interactions involving tapasin and the MHC class I HC has long been overlooked. This is likely due to the strong influence of the tapasin TMD on TAP stabilization. Nevertheless, it is now clear that the tapasin TMD impacts the assembly of MHC class I molecules independently from its role in TAP stabilization. Structural analyses as well as additional studies utilizing tapasin and/or MHC class I HC transmembrane mutants in the context of stable TAP levels will be valuable in defining the precise nature of transmembrane interactions within the loading complex.

Abbreviations

ER, endoplasmic reticulum; HC, heavy chain; MHC, major histocompatibility complex; TAP, transporter associated with antigen processing; TMD, transmembrane domain.

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Figure 1.

Transmembrane/cytoplasmic interactions within the MHC class I loading complex. The tapasin TMD associates with and stabilizes TAP1 and TAP2. For simplicity only the transmembrane helices of TAP I are shown here. The tapasin TMD may terminate in K408 (represented as a blue dot), allowing the remaining helix to bend upwards toward the ER membrane. This would position K408 in proximity to conserved arginine residues within the MHC class I transmembrane/cytoplasmic region (represented as two blue dots). The repulsive forces between charged residues may contribute to the destabilization of the tapasin/MHC class I HC association. Cysteine residues present in the cytoplasmic region of human tapasin and in some MHC class I molecules are shown as red dots. β_2m : β_2 -microglobulin.

Table I
Transmembrane/cytoplasmic regions of mouse and human tapasin

	393		394		395		396		397		398		399		400		401		402		403		404		405
Mouse	G		I		G		L		F		L		S		A		F		L		L		L		G
Human	S		V		G		L		F		L		S		A		F		L		L		L		G
	406		407		408		409		410		411		412		413		414		415		416		417		418
Mouse	L		L		K		V		L		G		W		L		A		A		Y		W		T
Human	L		F		K		A		L		G		W		A		A		V		Y		L		S
	419		420		421		422		423		424		425		426		427		428		429		430		431
Mouse	I		P		E		V		S		K		E		K		A		T		A		A		S
Human	T		C		K		D		S		K		—		—		—		—		—		—		—
	432		433		434		435		436		437		438		439		440		441		442		—		—
Mouse	L		T		I		P		R		N		S		K		K		S		Q		A		—
Human	—		—		—		—		—		—		—		K		K		A		E				

Sequences from the human and mouse tapasin transmembrane/cytoplasmic regions are shown with non-identical amino acids in bold. The amino acid residues are numbered using the first position after cleavage of the signal sequence as number 1. The tapasin transmembrane region has been predicted to include amino acid residues 393–417 or 393–407 (Ortmann et al., 1997), or 393–413 (Deverson et al., 2001), or 394–416 (Papadopoulos and Momburg, 2007).