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Review

Evolutionary and functional perspectives of the major histocompatibility complex class I antigen-processing machinery

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Abstract. Major histocompatibility complex (MHC) class I molecules present antigenic peptides to $CD8^+$ T cells, providing the basis for immune recognition of pathogen-infected cells. Peptides generated mainly by proteasomes in the cytosol are transported into the lumen of the endoplasmic reticulum by transporters associated with antigen processing (TAP). The maturation of MHC class I molecules is controlled by a number of accessory proteins and chaperones that are to a varying degree dedicated to the assembly of MHC class I. Several newly characterised proteins have been demonstrated to play important roles in this process. This review focuses on the functional relationship and evolutionary history of the antigen-processing machinery (APM) components and MHC class I itself. These are of great interest for further elucidating the origin of the immune system and understanding the mechanisms of antigen presentation and immunology in general.

Key words. Endoplasmic reticulum (ER); MHC class I; tapasin; TAP; ERp57; ERAP1/ERAAP; proteasome; antigen-processing machinery (APM).

Antigen presentation in the context of MHC class I

Major histocompatibility complex (MHC) class I molecules are constitutively expressed at almost all nucleated cells and present peptides of usually eight or nine amino acids in length to $CD8^+$ T cells [1]. If qualitative or quantitative changes in the presented peptide repertoire are detected, that potentially triggers an immune response, and the antigen-presenting cell will be eliminated. The quantitative changes of presented peptides are of particular importance for detection of malignant transformation of cells. The presented peptides are derived from both normal cellular and altered cellular proteins, or from intracellular pathogens, including viruses and bacteria. In cases of cross-presentation, extracellular pathogens may also be presented [2].

Peptides presented on MHC class I molecules are generated mainly in the cytosol by the action of proteasomes. These cleave proteins targeted for degradation into peptides with suitable carboxy-termini and of approximate length to fit into the MHC class I peptide-binding groove [3, 4]. Peptides are then imported into the endoplasmic reticulum (ER) across its membrane by transporters associated with antigen processing (TAP). This peptide transport process can be divided into two steps: an ATP-independent binding of cytosolic peptide to TAP [5, 6], followed by the energy-consuming translocation of peptide across the membrane [7–9]. Within the ER lumen peptides are loaded onto MHC class I heavy-chain (HC)-β2-microglobulin (β2-m) dimers. Evidence indicates that initially MHC class I molecules are loaded with suboptimal peptides that are subsequently replaced or trimmed, resulting in cell surface presentation of only high-affinity peptide-MHC class I complexes [10]. Indeed, not all the peptides transported by TAP are suitable for high-affinity

binding to MHC class I. However, the ER aminopeptidase associated with antigen processing (ERAAP), also named ER aminopeptidase 1 (ERAP1), is capable of trimming the amino-termini of certain peptides. This increases the binding affinity of these peptides to MHC class I and the fraction of ER luminal peptides available for stable presentation [11, 12].

The assembly and peptide loading of MHC class I is controlled and facilitated by a wide array of chaperones and assisting proteins working in concert (reviewed in [13, 14]), as illustrated in figure 1. The early stage of MHC class I maturation involves interaction with the general chaperone BiP, the ER lectins calnexin and calreticulin, and the thiol oxidoreductase ERp57 [15–18]. The dimerised HC-β2-m molecule may receive a high-affinity peptide at this stage, which will result in stabilisation and export to the cell surface without any need for further retention in the ER [19]. However, if the bound peptide has insufficient affinity to stabilise the complex, further quality control is needed. This late-stage maturation takes place in the peptide-loading complex (LC), which consists of tapasin, TAP, calreticulin and ERp57, and possibly other unidentified partners [20]. Tapasin is a dedicated MHC class I chaperone, binding and incorporating MHC class I into the LC [21]. It interacts with coat protein I (COPI) vesicles via a double lysine motif in its C-terminal cytoplasmic tail [21]. COPI vesicles are known to ferry cargo molecules in a retrograde fashion from the Golgi to the ER [22, 23]. A recycling mechanism of escaped, suboptimally loaded MHC class I molecules back into the ER in COPI-coated vesicles has been suggested as a mechanism for additional rounds of peptide optimisation [19, 21, 24]. The stability and cell surface expression of MHC class I is strictly dependent on proper folding and assembly of HC and β2-m and high affinity for MHC class I of the peptide [25]. Without the interaction with tapasin, MHC class I molecules loaded with low-affinity peptide are able to escape to the cell surface [26], where rapid degradation occurs due to low stability.

APM components are well conserved among species but have developed highly specific characteristics both between and within species

The adaptive immune system is considered to be absent in the jawless vertebrates and to have originated in an ancestor of the jawed fish, as defined by the presence of *bona fide* immunoglobulins, T cell receptors, MHC products and enzymes required to allow gene segment recom-

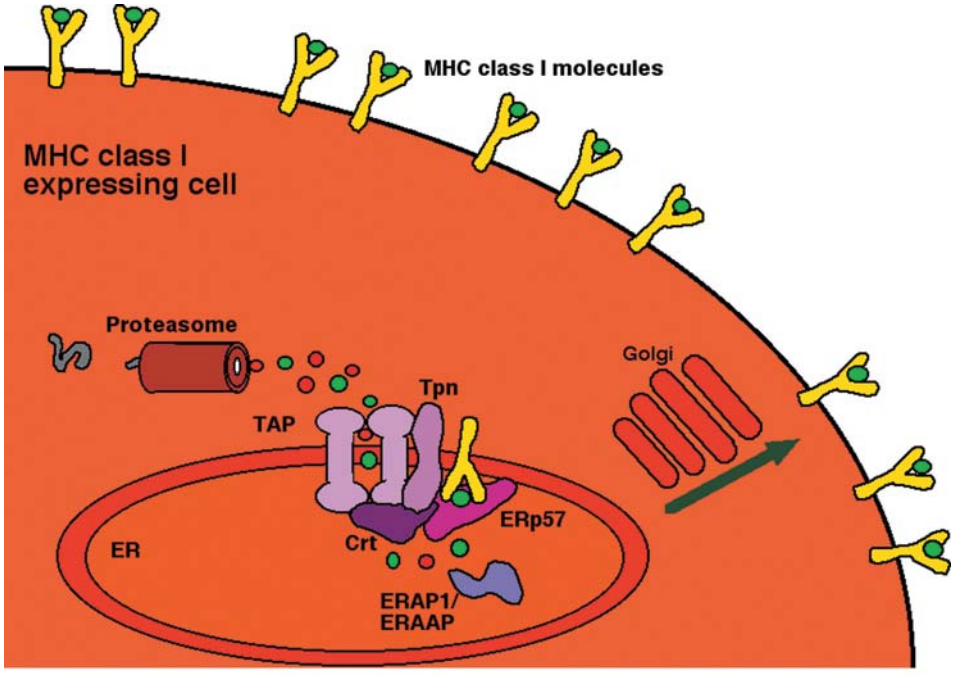

Figure 1. The components of the APM co-operate in the assembly of MHC class I-peptide complexes. Peptides generated by the proteasome are transported across the ER membrane by TAP. Newly synthesised MHC class I heavy chains (HCs) are initially associated with general ER chaperones including BiP and calnexin. After β2-microglobulin (β2-m) has bound the MHC class I heavy chain, tapasin binds this heterodimer. This bridges it to the loading complex (LC), which consists of tapasin, TAP, calreticulin and ERp57, and possibly other as yet unidentified proteins. In the LC, peptides with low affinity for the MHC class I binding groove are replaced with optimised peptides. Eventually the stable MHC class I-peptide complex is transported through the secretory pathway to the cell surface. ER, endoplasmic reticulum; Crt, calreticulin; Tpn, tapasin; TAP, transporter associated with antigen processing; ERAP1/ERAAP, ER aminopeptidase 1/ER aminopeptidase associated with antigen processing.

bination [27–29]. However, despite differences in immune defences, development of individual proteins and protein motifs in the vertebrate APM display a high degree of conservation across distant phyla.

Cell surface presentation of antigenic peptides in the context of MHC class I forms the basis for cellular immune defence against intracellular pathogens. The MHC class I antigen-processing machinery (APM), including at least the proteasome, ERAP1/ERAAP, TAP, general ER chaperones and the MHC class I maturation-specific accessory protein tapasin, is essential for MHC class I antigen presentation. This review will focus on APM components involved in the late stage of MHC class I maturation, rather than the chaperones that operate mainly during early stages of MHC class I maturation.

The proteasome

Cells have not evolved specific proteolytic systems for the generation of MHC class I-presented peptides. Instead they have co-opted catabolic proteolytic systems, phylogenetically dating far back. Using these proteolytic systems for the specific tasks of immune defence, specific regulatory proteins have evolved, including the interferon (IFN)-γ-inducible immuno-proteasome subunit low-molecular-mass polypeptides 2 and 7 (LMP2 and LMP7), multi-catalytic endopeptidase complex-like 1 (MECL1) and the proteasome activator PA-28α/β.

Proteasomes are large multi-subunit complexes with protease activity, and are found in organisms ranging from archaebacteria to higher eukaryotes. They are highly abundant within the cell and are heavily used for multiple purposes. In eukaryotes, 1% of the total cellular protein content is attributed to proteasomes within the cytosol and nucleus [30]. They are responsible for the main bulk turnover of proteins in the cell, thereby maintaining homeostasis between anabolic and catabolic pathways [31]. Moreover, ubiquitination targets misfolded proteins or proteins resulting from defective ribosomal processing for degradation by the proteasomes [30, 32]. In addition to the scavenger function, proteasomes have roles in cellular differentiation [33, 34], regulation of the cell cycle [35], control of neurotransmission strength by regulated degradation of presynaptic proteins [36], the cellular stress response and degradation of tumour suppressors and oncogenes [37].

A direct function for proteasomes in the generation of antigens for MHC class I presentation has been proven [38]. Almost a decade ago, Rock and colleagues demonstrated that proteasome inhibitors reduced antigen presentation by MHC class I molecules [31]. Today, the proteasome has been clearly demonstrated as essential for MHC class I antigen presentation. The degradation of cytosolic proteins is tightly regulated: if protein degradation is constitutively high, the energy loss, as well as the risk for induction of autoimmune disease, is increased. During microbial infection, both the rate and specificity of proteasome protease activity change, resulting in increased variety and size of the peptide pool suitable for MHC class I presentation (for review see [39]). Interestingly, the proteasomal breakdown of proteins from the normal turnover of cellular proteins is not the main source of peptides presented by MHC class I. Instead, most are generated from proteasomal degradation of defective ribosomal translation products (DRiPs) [40, 41]. The use of these newly translated proteins as a source for antigen presentation facilitates rapid recruitment of CD8+ T cells to virally infected cells, allowing their efficient destruction.

The simplest form of the proteasome is the cylindrical 20S complex. The 20S proteasome was observed using electron microscopy as early as 1968, and was initially called cylindrin [42]. Its proteolytic activity was demonstrated in the 1980s, thereby bringing the designation proteasome into use [43]. The 20S proteasome is built of four stacked heptameric rings: the two outer rings of proteins from the proteasome subunit α-family and the two inner rings of subunits from the β-family (fig. 2) [44, 45]. In eukaryotic proteasomes, protease activity is localised to three of the seven β-subunits at the interior of the barrel [46]. In this way, surrounding proteins are in no danger of degradation, since only the proteins shuffled directly into the proteasome are exposed to the proteolytically active β-subunits.

Phylogenetic analysis has revealed that the 20S α- and β-subunits originated by gene duplication of a single ancestral gene very early in evolutionary history, prior to the divergence of archaebacteria and eukaryotes [47]. Despite being present in both prokaryotes and eukaryotes, the functions and targets of the proteasome differ between phylogenetic domains. In prokaryotes, redundancy of proteolytic systems has been suggested to account for the dispensable nature of proteasomes [48]. Higher eukary-

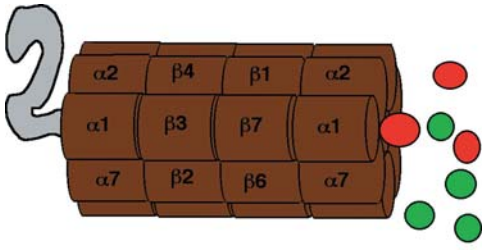

Figure 2. The structure of the 20S proteasome shows high evolutionary conservation. The 20S proteasome is cylindrical. The two outer rings lack proteolytic activity and are made of α-subunits. They create a pore of approximately 0.13 nm in diameter. The proteolytic activity of the β-subunits is buried within the interior of the proteasome and is only exposed to proteins shuffled into the barrel. Proteins targeted for degradation are shuffled into the proteaseome, and peptides are generated.

otes (mammals) have evolved specific proteasome components allowing the usage of the proteasomes for specific actions. Generation of MHC class I binding peptides is regulated by activator complexes that bind to the ends of the 20S proteasome barrel, forming the 26S proteasome [49–51]. One of these regulatory complexes is the activator PA28 [52, 53]. PA28α/β is induced by IFN-γ. Simultaneously, the constitutively expressed regulator, PA28γ, is downregulated, enhancing presentation of certain viral antigens independent of the subunit composition of the 20S proteasome [39, 54].

Multiple gene duplications in eukaryotes have given rise to large families of proteosomal α- and β-subunits [47], the latter including the specific immuno-proteasome β-subunits present in mammals. Immuno-proteasomes are formed by substitution of the three catalytically active subunits β1, β5 and β2 with three related β-subunits, LMP2, LMP7 and MECL1 [55]. *LMP2*, *LMP7* and *MECL1* were formed by gene duplication events occurring prior to the divergence of lamprey from jawed vertebrates [47], and are all IFN-γ inducible [56]. Both *LMP2* and *LMP7* are located in the MHC II region on chromosome 6 [57], whereas MECL1 is encoded on a presumably paralogous region on chromosome 9 [58]. IFN-γ also post-transcriptionally downregulates the constitutively expressed β-subunits [59, 60]. This changes proteasome specificity towards cleavage after basic and hydrophobic residues [61, 62]. Interestingly, TAP and many MHC class I molecules preferentially bind peptides with hydrophobic carboxy-termini. The exchange of constitutively expressed β-subunits with immuno-subunits therefore increases the amount of peptides suitable for presentation by MHC class I [63]. Increased numbers of peptides available for MHC class I presentation during infection may also result from the fact that proteasomes in close proximity to the ER incorporate LMP2 and LMP7 to a greater degree than proteasomes elsewhere in the cell [64]. Most peptides generated by proteasomes have sizes ranging from 6 to 11 amino acids, although a substantial number of longer peptides are also generated [4, 65]. Consequently a large fraction of proteasome generated peptides meet the peptide size preference of TAP, which preferentially transports peptides of 8–16 amino acids in length [66–68].

The immuno-subunits have been proposed to direct the focus of antigen processing towards responding to pathogens, and to limit temporarily the production of self-peptides. Indeed, the expression of immuno-subunits increases the presentation of antigenic peptides in the context of MHC class I during infection with several different types of viruses, including influenza A, adenovirus type 5, hepatitis B and lymphocytic choriomeningitis virus [54, 69–71]. Conversely, the change in protease substrate specificity might decrease the generation of certain other viral antigens. This flexibility to alter the range of antigens presented at the cell surface enables the immune system to react to a more diverse set of infectious agents.

ERAP1/ERAAP

The function of the proteasome as a carboxypeptidase for generation of MHC class I binding peptides has been known for over 10 years. The presence of an aminopeptidase involved in this process until recently remained elusive, when two groups independently identified and characterised an enzyme named ERAP1 or ERAAP. ERAP1/ERAAP is localised to the ER lumen, has its expression regulated by IFN-γ and trims MHC class I-binding peptides [12, 72]. It was previously characterised as a metallopeptidase named adipocyte-derived leucine aminopeptidase (A-LAP) [73] or puromycin-insensitive leucyl-specific aminopeptidase (PILS-AP) [74].

MHC class I presentation of several peptides is directly influenced by ERAP1/ERAAP, as shown by the effect of small interfering RNA knockdown of ERAP1/ERAAP expression in living cells. Expression of ERAP1/ERAAP increases the total pool of MHC class I at the cell surface. The absence of ERAP1/ERAAP modulates peptide presentation: some peptides were presented to a greater degree, presentation of other peptides was abolished and some peptides were unaffected [12, 72]. IFN-γ upregulates ERAP1/ERAAP, in concordance with its regulation of several other APM components as summarised in table 1 [12, 72]. The peptide length preferences between TAP and MHC class I differs: TAP preferentially transport peptides of 8–16 amino acids [66–68] and MHC class I bind peptides of 8–10 amino acids in length [75]. This discrepancy is partially compensated by the amino-terminal trimming of peptides in the ER by ERAP1/ERAAP. Interestingly, efficient proteolytic removal of amino-terminal peptide residues in the ER only takes place in the presence of appropriate MHC class I molecules [76], and it was suggested that ERAP1/ERAAP is

Table 1. IFN-γ regulates the expression of the APM components. IFN-γ increases the expression of several APM proteins and reciprocally downregulates constitutively expressed counterparts.

Upregulation by IFN-γ	Down-regulation by IFN-γ
HC	
β2-m	
LMP2	β1
LMP7	β5
MECL1	
PA28α	PA28γ
PA28β	
TAP1	
TAP2	
Tapasin	
ERAP1/ERAAP	

integrated directly into the LC [77]. ERAP1/ERAAP has so far been characterised only in the human, mouse and rat. Cloning and characterisation in other species as well as further comparative studies will reveal whether there are species specificities in its aminopeptidase activity, such as those seen in the proteosomal peptide generation of HLA-B27-presented peptides [78].

TAP

TAP was cloned in the early 1990s [79–82]. Peptide translocation from the cytosol to the ER lumen by TAP is a two-step process, in which ATP-independent peptide binding is followed by ATP-consuming transport. The exact mechanism behind these two steps still remains to be elucidated. The heterodimeric TAP consists of TAP1 and TAP2. The ~35% identity at amino acid level between TAP1 and TAP2 in species in which it has been sequenced so far indicates that *TAP1* and *TAP2* probably have arisen by duplication from a common ancestral gene [83]. The human *TAP* genes are located in the MHC class II region at chromosome 6, in close proximity to the genes coding for proteasomal immuno-subunits LMP2 and LMP7. The TAP proteins are members of the ATP-binding cassette (ABC) superfamily, comprising a diverse set of membrane proteins that transport a broad set of molecules in an ATP-dependent manner [84, 85]. The ABC family is one of the largest protein families, and members are defined by the presence of a conserved ABC unit in the nucleotide-binding domain (NBD). The highly conserved NBD harbours Walker-A and -B motifs, as well as a LSGGQ signature motif [84]. Mutation of TAP1 or TAP2 in either of the Walker motifs or in the signature region results in impaired function due to abolishment of ATP-dependent peptide translocation across the ER membrane [86–88].

In the rat, different TAP2 alleles confer substrate specificity in terms of the peptides transported across the ER membrane. The polymorphism in rat TAP2 referred to as class I modifier (cim) polymorphism results in distinct peptide loading efficiencies of different rat MHC class I molecules (RT1.A), giving rise to differences in epitopes presented at the cell surface. The cim^b alleles, b and u, show specificity for transport of peptides with hydrophobic carboxy-termini. This feature is shared by mouse TAP. Meanwhile, the cim^a alleles, a and l, are similar to human TAP, since they are more promiscuous and transport peptides with either hydrophobic or basic carboxy-termini [89]. These studies are in agreement with the proposal that the linkage of different RT1.A molecules with cim variants of TAP2 have co-evolved for functional reasons [90] and that conservation of specific combinations of TAP2 and RT1.A alleles on haplotypes is advantageous for loading of stabilising peptides to the RT1.A peptide-binding groove [91].

Human TAP is less polymorphic than rat TAP, but there is nevertheless variation. A splicing variant called TAP2iso, co-expressed with normal TAP2 in human lymphocyte cell lines [92], is functional and restores cell surface expression of MHC class I when expressed with TAP1 in the TAP-deficient cell line T2. The specificity of the TAP1-TAP2iso dimer expressed in T2 cells was demonstrated to differ significantly to that of the TAP1-TAP2 dimer when studied for a set of peptides [92]. Moreover, single nucleotide polymorphisms (SNPs) in TAP have been identified, and these may serve as markers for specific diseases, including ankylosing spondylitis and Graves' disease [93].

ERp57

ERp57 is a thiol-oxidoreductase with two characteristic thioredoxin CGHC motifs (TR1 and TR2) and a C-terminal QEDL retention signal for soluble ER proteins [94]. In contrast to the archetypal protein disulphide isomerase (PDI), ERp57 interacts specifically with monoglucosylated glycoproteins [95, 96]. This binding is mediated by either one of the two ER lectins, calnexin or calreticulin [97].

Recently, ERp57 was demonstrated to be involved in the maturation of MHC class I [17, 98–100]. ERp57 binds MHC class I HC during early stage assembly, independent of β2-m [17]. This interaction appears to depend on ER lectins. ERp57 also associates with MHC class I during late-stage assembly as part of the LC and this interaction does not require lectins [17]. Instead, ERp57 covalently binds to tapasin as a result of a labile disulphide bond formed between cysteine 57 in the TR1 of ERp57 and cysteine 95 (Cys95) in the flexible region of the tapasin luminal domain [100]. The ERp57-tapasin conjugate is dependent on the presence of MHC class I. Abolishment of the ERp57-tapasin interaction through mutation of the Cys95 results in unstable MHC class I molecules with an altered peptide repertoire expressed at the cell surface. In contrast to mutation of Cys95 in tapasin, mutations of either of the two more N-terminal cysteine residues (Cys7 and Cys71) did not completely abrogate the conjugation of ERp57 and tapasin. A significant reduction, however, of the number of ERp57-tapasin conjugates was observed, compared to cells expressing wild-type tapasin. The surface expression of HLA-B44 was also reduced to levels similar to those seen in tapasin Cys95 mutant cells. In cells expressing wild-type tapasin, only fully oxidised HLA-B44 was detected in the LC, whereas expression of any of the tapasin cysteine mutants resulted in detection of partially reduced HLA-B44.

Several studies have indicated that complete oxidation of the HC is a prerequisite for binding to the LC [101–104]. The MHC class I HC has two disulphide bonds, one in the membrane-proximal α-3 domain and the other in the α-2 domain that forms part of the peptide-binding cleft. The interaction of MHC class I with tapasin-ERp57 conju-

gates has been suggested to depend on the α-2 domain cysteine residues at positions 101 and 164 (Cys101 and Cys164) [104]. Cys101 and Cys164 are fully conserved in all known MHC class I molecules. The redox status of MHC class I molecules has been shown to be influenced by ERp57-tapasin conjugates, and a role for isomerisation of the HC α-2 disulphide bond during late-stage maturation has been suggested [100]. This isomerisation requires further study, but it might allow for the replacement of low-affinity peptide in the LC. A role for ERp57 in reducing intrachain disulphide bonds in MHC class I molecules targeted for degradation was inferred from a study where recombinant ERp57 was shown to reduce partially folded MHC class I in vitro [105].

The HC disulphide bond formed by cysteine 203 and 259 contributes to a classical immunoglobulin (Ig) fold. The importance of disulphide bonds in the Ig fold is indicated both by functional studies (for review see [106]), but also from the high degree of conservation of this feature in the Ig fold family (IgFF) [107]. This is a heterogeneous family, defined by the presence of the Ig-fold composed of two sheets of anti-parallel β-strands linked by a disulphide bond. The proteins of the IgFF are distributed in various tissue types and represented throughout viruses, prokaryotes, fungi, plants and higher eukaryotes [108]. Since the family has a very low level of mean sequence identity, the evolutionary mechanisms behind the Ig-like fold have been difficult to determine, although subgroups, such as the Ig family, share a common phylogenetic history [109]. The necessity of disulphide bonds for achieving the correct tertiary structure of Ig-like domains has been questioned, since not all IgFF members depend on oxidation during folding [110]. Instead of being central to the folding pathway, it was suggested that the disulphide bond in the Ig fold has a more important functional role in the protein.

The requirement for correct disulphide bond formation in MHC class I HC during maturation has been clearly demonstrated [100]. Mutation of either of the α-2 or α-3 disulphide bonds abrogates cell surface expression and leads to ER accumulation of misfolded HC [101, 111, 112]. MHC class I maturation and the role of ERp57 in this process will surely be studied further, and the results will be of great interest. The redundancy of cellular thiol oxidoreductases may compensate for a lack of ERp57 during MHC class I maturation at early stages [113]. In light, however, of the conjugation of tapasin-ERp57 in the LC and the instability of cell surface-expressed MHC class I molecules in the absence of ERp57-tapasin conjugates, a requirement for ERp57 during late stage MHC class I maturation is indicated [100].

Tapasin

Tapasin is a type I transmembrane protein with a large N-terminal ER luminal region, a single transmembrane-spanning domain and a short cytoplasmic tail [114–116]. Tapasin was shown to bind stably to TAP in equimolar ratios [114], but complexes of as many as four tapasin molecules per TAP have been reported [115]. Tapasin is a dedicated MHC class I chaperone, binding transiently to MHC class I molecules and thereby incorporating them into the LC [114]. In the absence of tapasin, MHC class I is not found in association with TAP [117]. Tapasin-deficient cells have decreased cell surface expression of MHC class I, with alterations in their presented peptide repertoire and impaired cytotoxic T lymphocyte (CTL) response [117–120].

The origin of the *tapasin* gene is an intriguing question. *Tapasin,* together with the genes for several of the APM components, maps to the same chromosomal region of the MHC class II locus in all jawed vertebrates tested to date [121]. It has been suggested that tapasin and MHC class I molecules share a common evolutionary history based on similarities in amino acid sequences, predicted tertiary structure and domain organisation, and in exon-intron organisation of the encoding genes [122]. The three-dimensional structure of tapasin remains unsolved and awaits successful NMR and crystallographic studies. These should contribute considerably to the understanding of *tapasin* evolution and will be of great importance for understanding its function as well as the overall organisation of the LC.

Biochemical and biophysical studies of recombinant soluble tapasin have provided valuable clues as to its structural organisation. Among other features, they suggest the existence of two stable luminal core regions [123]. The N-terminal core domain is highly rich in prolines and has a conserved cysteine pair, thought to form an intramolecular disulphide bridge [100]. Together with a short flexible linker region, the N-terminal core constitutes an ~100-amino acid-long domain with no homology to known protein sequences. The membrane-proximal luminal core domain is ~300 amino acids long and has been predicted to resemble the three-dimensional structure of the MHC class I HC [122]. This core domain includes a partial Ig-like domain followed by a domain with no known homology, with the exception of chicken tapasin, in which this domain contains a proposed modified Ig fold [124]. The most membrane-proximal part of this domain contains an Ig-like domain with another two conserved cysteines, suggested to form an Ig-fold disulphide bond [125].

Tapasin has also been suggested to be related to the MHC class II-like molecule HLA-DM based on similarities in gene organisation [125]. Future crystallographic comparison of HLA-DM and tapasin will shed light on the structural and functional parallels between these two molecules. Striking differences in organisation of the *tapasin* gene from both MHC class I as well as MHC class II genes have been noted [122]. In *tapasin,* the signal sequence is interrupted by an intron, an uncommon feature in Ig su-

perfamily members. The signal sequence interruption is, however, shared by a specific subset of the IgFF, including FcαRI [126] and the killer-cell inhibitory receptors (KIRs) [127]. An analysis of the evolutionary relationship of the tapasin Ig domain to other Ig domains suggested that tapasin is most closely related to β2-m and the MHC class I-like protein Zn α-2 glycoprotein [125]. A common ancestor to tapasin and other members in the MHC region is implied by analysis of the *tapasin* gene structure. With respect to the unique organisation of the *tapasin* gene, a possible model for the evolution of tapasin is that it has risen from not one single gene but from several. Figure 3 shows the organisation of the *tapasin* gene in the MHC region at human chromosome 6.

Tapasin is highly conserved between species studied so far. Human tapasin shows 75% identity at the level of the primary sequence to the murine and rat equivalents (which show 85% identity to each other). In addition, there is a high proportion of conserved substitutions between tapasin from different species [128]. The hydrophobicity profile is also well preserved, implying similarities in the three-dimensional structures. Not only are many features of tapasin well conserved between mammals, but these also extend to species as distantly related as birds and fish. One such feature is the double lysine motif in the carboxy-terminus of tapasin; another is the two cysteine pairs suggested to form the disulphide bonds in the Ig folds, both of which are found in all species studied to date [129]. An N-linked glycosylation site is located in the ER luminal region of tapasin in all species studied to date, with the exception of chicken tapasin [124].

Recently, a novel protein was characterised and named tapasin-R (tapasin-related). Tapasin-R is a member of the Ig superfamily and encoded on chromosome 12p13.3, a region suggested to be paralogous to the MHC [130]. Tapasin-R shares several structural features with tapasin, and the intron-exon organisation suggests a common ancestor. Differences that might contribute specificity to these two related proteins include the lack of double lysine motif in tapasin-R. Probably as a result of the lack of this ER retrieval signal, ~10% of tapasin-R is found at the cell surface, compared to tapasin that is located exclusively in the secretory pathway [21, 130].

Studies aiming to elucidate the function of tapasin in different species have revealed both similarities and differences. The role of tapasin to bridge MHC class I to the TAP complex is conserved between species and well proven [114, 116]. MHC class I molecules expressed in the tapasin-deficient human cell line 721.220 [117, 131, 132] or in tapasin knockout mice [119, 120] do not interact with TAP. H2-L^d, -K^d and -K^b transfected into the tapasin-expressing cell line 721.221 were all able to bind to the human TAP complex and were efficiently expressed at the cell surface [133]. Transfection of either murine or human tapasin complementary DNA (cDNA) into HLA-B5 and B8 expressing 721.220 cells was shown to restore both the TAP-HLA-B5 and -B8 interactions, as well as the cell surface expression of these HLA molecules [128]. However, another study demonstrated that co-transfection of HLA-B*4402 and mouse tapasin cDNA into 721.220 cells resulted in a very low proportion of the expressed HLA-B*4402 linked by mouse tapasin to the human TAP complex [134]. Yet another study with HLA-B*4402-transfected J26 cells (mouse L cell line expressing human β2-m) showed that constitutively expressed murine tapasin could bind HLA-B*4402 and link this human MHC class I to the murine TAP complex, although peptide loading and surface expression were impaired [135]. Using other murine cell lines confirmed the impaired cell surface expression of HLA molecules in mouse cells [135]. Restored maturation and cell surface expression of HLA-B*4402 was achieved by transfection of human tapasin cDNA into these cells. Overexpression of mouse tapasin in J26 cells also resulted in restoration of HLA-B*4402 expression and presentation of viral antigens to CTLs [135]. Since the en-

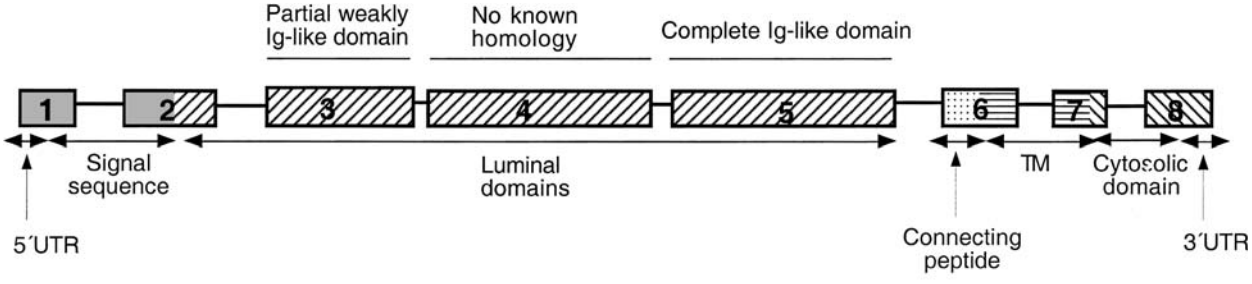

Figure 3. Schematic presentation of the tapasin intron-exon organisation. The tapasin gene encompasses eight exons. The signal sequence is encoded by exon 1 and part of exon 2. Signal sequence interruption is uncommon in Ig superfamily members, but is shared by a specific subset of the IgFF, including FcαRI and the killer-cell inhibitory receptors. A partial, weakly Ig-like domain in exon 3 encodes together with exons 4 and 5, and part of exon 6, the tapasin ER luminal domains. Exon 4 has a predicted structure of no known homology, whilst exon 5 encompasses a complete Ig-like domain. Exon 6 encodes a connecting peptide and the amino-terminal part of the transmembrane domain. The remaining transmembrane domain is encoded in exon 7, and exon 8 encodes the cytosolic domain and to some extent the 3′-untranslated region. UTR, untranslated region; TM, transmembrane domain.

dogenous tapasin in mouse cells is not sufficient to ensure proper maturation of HLA, these results indicate a species difference between mouse and human tapasin. HLA-B*2705 is known to be one of the alleles with low dependence on tapasin for maturation. The expression of HLA-B*2705 in J26 cells did not require co-expression of human tapasin for maturation. Studies of HLA-B8 demonstrated that it is intermediate in dependence on human tapasin for proper maturation in J26 cells, compared to HLA-B*4402 and -B*2705 [136]. These experiments show that mouse tapasin is not fully able to replace human tapasin in terms of the quality improvement of highly tapasin-dependent alleles such as HLA-B*4402, and that the variation in the tapasin-dependence spectra for HLA alleles is significant.

Functional comparison of MHC class I maturation in the presence of tapasin from different species suggests species-specific characteristics [116]. Analysis of its structure indeed reveals some distinct features. Tapasin bears only one potential glycosylation site in species studied hitherto with the exception of rat tapasin, which has two potential glycosylation sites [129]. However, the tapasin glycosylation sites have so far not been proven to play any functional role in MHC class I maturation. One feature that is more likely to account for species specificity is the C-terminal cytoplasmic tail. In rodent tapasin, it is 14 amino acids longer than the human equivalent, and the identity at the amino acid level is only 50%. Recently, a 9 amino acid region in the Ig-like domain of tapasin was demonstrated to dictate the affinity of human, but not mouse, tapasin for H-2L^d [137]. The differences between tapasin in various species are suggested from both in vivo and in vitro studies [119, 120]. Importantly, the tapasin deficiency in the 721.220 cell line was generated by γ-irradiation [138] and therefore it has to be taken into account that this cell line might have uncharacterised mutations of importance for MHC class I antigen presentation.

MHC class I

Different MHC class I alleles exhibit different degrees of dependence for tapasin and possibly other components of the APM for maturation [117, 131, 133]. As mentioned above, certain alleles, such as HLA-B*2705, present antigenic peptides at the cell surface even in the absence of tapasin interaction [78, 118, 136, 139]. In the presence of tapasin, however, the stability of peptide-HLA-B*2705 complexes are significantly increased by the different peptide repertoire presented. Presentation of antigenic peptides in a tapasin-LC-independent manner has been speculated to have evolved as a mechanism for the immune response against viral infections targeting the LC [140]. The differences in dependence on association with tapasin and the LC may also contribute to broadening of the range of possible antigens presented in the context of MHC class I molecules. Further studies where different alleles are studied in parallel will drive the field towards a clearer understanding of the role of tapasin in general, as well as in the maturation of specific MHC class I alleles.

Cell surface expression and stability of MHC class I allelic products differ significantly in terms of tapasin dependence [132, 133, 136, 138, 141]. The question of how MHC class I polymorphism influences the variation in interaction with the LC during class I maturation is of great interest. First, the intrinsic stability of MHC class I allelic products varies and may contribute to different degrees of tapasin dependence as well as to other dissimilarities in chaperone interaction patterns [142, 143]. Second, natural MHC class I polymorphisms as well as specific single amino acid mutations significantly affect its binding to the LC. Mutational analyses have proven that most of the solvent-exposed α-2 loop, comprised of residues 128–137, in H-2L^d is involved in tapasin-LC interaction [144]. The same α-2 loop is essential for tapasin interaction in humans, since the HLA-A2 mutant T134K does not interact with the LC [145, 146]. Other non-polymorphic residues implicated in the interaction between the HC and tapasin-TAP include the α-3 loop residues 219–233, as indicated from studies of H-2L^d, H-2D^b and HLA-B*2705 [147–149]. The polymorphic residue 116 in the HC is located in the peptide-binding groove and has been shown to influence the binding of MHC class I to tapasin (reviewed in [150]). The pattern is complex, however, as illustrated by the polymorphic variants HLA-B*4402 and HLA-B*4403, which differ only at a single residue, 156. This single amino acid change significantly impairs the bridging of HLA-B*4403 to TAP, compared to HLA-B*4402 [151].

Certain MHC class I alleles are less stable than others. In mice this may be correlated to the different affinities of H2 alleles for β2-m: the less stable alleles H-2L^d and H-2D^b have lower affinities than the more stable alleles H-2D^d and H-2K^b for murine β2-m [152, 153]. Amino acid residue 9 was specifically evaluated and compared between these variants and was demonstrated not only to play an important role in this interaction but also to influence loading and presentation of antigenic peptides, both qualitatively and quantitatively [152]. When expressed in human cells, human and mouse MHC class I HCs differ in maturation, and this has been suggested to result from the greater stabilising effect of human β2-m on H2 HCs, compared to murine β2-m. Human β2-m has higher affinity than mouse β2-m for mouse HCs (H2 chains) [154], and cell surface expression of peptide-receptive murine MHC class I molecules is stabilised by the addition of exogenous human β2-m [152, 155]. Another variable that may contribute to the difference in maturation of H2 and HLA HCs is the location and number of glycosy-

lation sequons [156]. These are likely to dictate the dissimilarity in the interaction of the ER lectins calnexin and calreticulin with HCs from different species, as reviewed in [14]. The H2 HC has two glycosylation sequons allowing calnexin to bind first to the most N-terminally located sequon and then subsequently the more C-terminal sequon [157]. In this way the interaction with calnexin is prolonged for H2 HC compared to HLA HC, which contains only one glycosylation sequon (corresponding to the most N-terminally located seuqon in H2). Translocation of the N-terminal part of the nascent HLA HC results in initial calnexin binding. This is later abrogated when the membrane-bound calnexin is no longer topologically able to reach the sequon. Instead, the soluble homologue calreticulin replaces calnexin and binds to the maturing HLA molecule.

It is probable that the MHC class I and class II loci originated from a gene duplication event in early vertebrate history [158]. Exon rearrangements took place during this event as indicated by the different structures of MHC class I and II loci [159]. Thereafter, the MHC class I and class II loci have frequently undergone duplication events [160]. The process of gene duplication and deletion in mammals has been faster in MHC class I than in MHC class II [161, 162] and have resulted in no orthologous relationships between MHC class I loci in different orders of mammals [161] (two genes are said to be orthologous if they diverged after a speciation event [163]). Neither are orthologous relationships known for MHC class II loci from different classes of vertebrates, although they have been shown for mammals of different placental orders [162]. Gene duplication and deletion are not the only genetic events that have influenced the evolution of the vertebrate MHC. Evolutionary history reveals processes such as functional divergence of duplicated genes, mutational gene silencing, and intra- and interlocus recombination [164].

The MHC loci are highly polymorphic, and many of these polymorphisms have been preserved since the divergence of primate species [165–167]. This phenomena, called trans-species polymorphism, indicates that these variants are selectively maintained [165, 168]. The highly polymorphic nature of the MHC is striking and different theories have been proposed to explain this phenomenon [169]. Initially, a high mutation rate was suggested to account for the high degree of polymorphism. It is now known, however, that the mutation rate in mammalian MHC loci is not higher than the average locus mutation rate [170]. Another possibility for generating high diversity was suggested to be intra- and inter-locus recombination events. Both these recombination events are indeed part of the evolutionary history of the MHC. Recombination itself is, however, not a driving force for increased polymorphism. Only by combining selection favouring heterozygotes with recombination can increased diversity be generated. Heterozygote advantage may be considered a key principle to explain the diversity of many MHC loci. Suggested selective mechanisms for maintenance of the diversity include maternal-fetal interaction (increased fetal survival hypothesised to result from positive effects of maternal antibodies against fetal MHC class I), negative-assortative mating (individuals with different alleles mate more frequently than those sharing higher numbers of alleles) and, most important, parasite resistance [171].

Variation between MHC class I alleles is concentrated in the α-1 and α-2 domains. Analysis of amino acid residue heterozygosities in the peptide-binding site in HLA revealed around 10 times higher frequency than in regions not interacting with peptide or the T cell receptor [172]. Moreover, in both mice and humans, the peptide-binding region was found to have a higher rate of non-synonymous compared to synonymous substitutions [170, 173, 174]. This is not the case in the rest of the MHC molecule where the nucleotide substitution situation is reversed. Together, these results indicate the importance of heterozygote advantages for the maintenance of the highly polymorphic nature of the MHC loci and genetic variation in the peptide-binding region of MHC class I.

Perspectives and conclusions

The adaptive immune defence of jawed vertebrates has evolved independently of other phylogenetic groups [175]. The use of proteins and protein complexes can, however, be conserved between distant phyla. This demonstrates the phenomenon known as co-option or gene recruitment: the ability of organisms to evolve specific and novel functions for proteins through specialisation of genes [176]. In addition, use of regulatory proteins that modify the function of existing systems makes it possible for ancient systems to be used for new adaptations. A paradigm is the proteasome subunits that are distributed in organisms ranging from archaebacteria to mammals but that exert specialised biological functions in each group of organisms [177]. Moreover, species-specific 20S proteasomal cleavage of HLA-B*2705 presented peptides, between species as closely related as mice and humans, has been demonstrated [78]. This suggests that the proteasome contributes to the differences between species in the repertoires of MHC class I-presented epitopes.

Local DNA regulatory elements together with chromatin remodelling of specific regions influence the regulation of defined gene clusters. The human genes for tapasin, TAP1 and TAP2, and LMP2 and LMP7 are all located at chromosome 6 in the MHC class II cluster. Their close proximity suggests possible co-evolution of these MHC class I APM components. Co-regulation of the transcription of APM genes would be beneficial both under resting cellular state and infection.

Comparison of the amino acid sequence of peptides processed by the APM components at different levels also suggests co-evolution of several of the APM components. The proteasome generates peptides with basic or hydrophobic C-terminus. Human TAP has a preference for peptides with such residues in the carboxy-terminus and therefore readily translocates a large proportion of proteasome-generated peptides across the ER membrane. Many MHC class I molecules bind preferentially to peptides with hydrophobic and basic carboxy-terminus. Conversely, the binding preference for the amino-terminus of peptides differs between TAP and MHC class I. Since TAP, however, transports many peptides slightly longer than those of preferred length for MHC class I binding, these amino-terminus binding preferences do not have to be the same, in the presence of an efficient ER aminopeptidase. Recent studies have demonstrated that there is indeed an ER aminopeptidase, ERAP1/ERAAP, involved in MHC class I peptide presentation. This enzyme cleaves amino-terminal lysine (K), leucine (L), tyrosine (Y) and asparagine (N) residues, generating peptides of 8–9 amino acids [12]. The N-terminal residues lysine (K), asparagine (N) and arginine (R) are the most favoured by TAP for peptide transport, indicating a connection between the peptides provided by TAP to the ER lumen and the substrate specificity of ERAP1/ERAAP [178].

The ultimate goal of the MHC class I system is to present the peptide at the cell surface to scanning $CD8^+$ T cells. Up to two-thirds of the contacts between the peptide-MHC class I complex and the T cell receptor are mediated by the MHC class I itself [179]. The most variable part of the T cell receptor are the CDR3 loops interacting mainly with residues in the central part of the peptide [180, 181]. These peptide residues are localised in the most polymorphic part of the MHC class I molecule and under the least pressure for conservation for interaction with TAP and the MHC class I molecules *per se*. Variability in the peptide-T cell receptor-interacting region allows the immune defence to be highly specific and detect intracellular changes of many different kinds. Co-ordination of APM protein preferences for peptide termini, the physical localisation of APM genes and their upregulation by IFN-γ all confer efficiency in the processing of MHC class I-presented antigens.

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