Review

Chaperones and folding of MHC class I molecules in the endoplasmic reticulum

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Abstract

In this review we discuss the influence of chaperones on the general phenomena of folding as well as on the specific folding of an individual protein, MHC class I. MHC class I maturation is a highly sophisticated process in which the folding machinery of the endoplasmic reticulum (ER) is heavily involved. Understanding the MHC class I maturation per se is important since peptides loaded onto MHC class I molecules are the base for antigen presentation generating immune responses against virus, intracellular bacteria as well as tumours. This review discusses the early stages of MHC class I maturation regarding BiP and calnexin association, and differences in MHC class I heavy chain (HC) interaction with calnexin and calreticulin are highlighted. Late stage MHC class I maturation with focus on the dedicated chaperone tapasin is also discussed.
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1. Introduction

The maturation of MHC class I is interesting both from a biochemical point of view, with regards to the principles for protein folding, as well as from an immunological perspective, in view of the correct and stable presentation of antigenic peptides. MHC class I molecules are expressed on almost all nucleated cells of the body and present antigenic peptides to CD8^{+} T cells [1,2]. The peptides are derived from proteasomal degradation of proteins synthesised inside the cell, both from normal cellular proteins and from intracellular bacteria and viruses [3,4]. The ability of CD8^{+} T cells to recognise changes in the peptide repertoire expressed at the cell surface is essential for the immune defence against viruses, intracellular bacteria as well as against tumours [5,6]. The three components of MHC class I, the heavy chain (HC), β2-microglobulin (β2-m) and the peptide, are all essential for the formation and stability of a functional MHC class I [7,8]. The maturation and assembly of the MHC class I complex is complicated, and involves many steps including both peptide optimisation and several quality control steps. The folding of small soluble proteins has been studied extensively, but the more complex the protein, the more difficult the folding has been to address and understand. However, important information about the principles of transmembrane protein folding has been gained from in vitro and in vivo studies of viral proteins such as the Vesicular Stomatitis Virus Glycoprotein (VSV G protein) [9–13], as well as from studies of MHC class I. The knowledge achieved from such in vitro and in vivo folding studies of different substrates can be integrated and used to understand the general maturation of any kind of newly synthesised protein as well as revealing the specific maturation mechanisms needed for individual proteins such as MHC class I. Class I maturation involves interactions with classical chaperones, substrate-feature specific chaperones, catalysing enzymes as well as with MHC class I dedicated accessory proteins [14–21]. Recently, several new molecules affecting the MHC class I maturation have been identified (e.g. tapasin, ERp57 and ERAAP). These proteins have only been studied to a limited extent but it is already clear that they are intimately involved in the maturation of class I. The information received from studies of these newly discovered accessory molecules, together with the knowl-

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edge of other accessory molecules and folding in general, help in the understanding of MHC class I maturation.

2. In vitro and in vivo folding

The initial hypothesis that the native conformation of a protein is the thermodynamically most favourable polypeptide state is today known to be a very simplified view. This is illustrated by comparing folding of simple proteins with more complex ones as well as by comparison of in vitro and in vivo folding. In small globular proteins, the amino acid sequence does not need any help for formation of the secondary structures in vitro [22]. This folding is very efficient and presumably driven by hydrophobic forces. The α-helices and β-sheets may then interact with each other to form the native tertiary structure. Achieving this state in the test tube is simplified by the controlled milieu allowing low protein concentration and low temperature. However, regulation of temperature and protein concentration does not seem to be sufficient for efficient folding of more complex proteins. Spontaneous folding in vitro has been observed only for a limited number of proteins, such as hen lysozyme [23]. Furthermore, the majority of these proteins that fold efficiently in vitro are small, single-domain proteins, indicating the need for other factors to control the folding of multi-domain proteins.

Protein folding in the cell takes place either in the cytoplasm (cytoplasmic proteins) or in the endoplasmic reticulum (ER) (transmembrane and secretory proteins). There are differences between folding in the ER and the cytosol but also remarkable similarities [24–26]. However, this review will deal with ER folding only. Folding of newly synthesised proteins in the ER differs significantly from folding in vitro [27]. The first reason for this is the very high protein content in the ER [27–30]. The high concentration increases the intermolecular association constant and makes nascent polypeptides prone to aggregate. A second reason is the nature of the entry of translated proteins into the ER. The majority of nascent polypeptides are translated with the N-terminus entering the ER first so that the N-terminal part of the polypeptide becomes available for folding as well as aggregation before the rest. However, aggregation and misfolding during vectorial insertion of polypeptides, which are still being translated upon ribosomes in vivo, is reduced since domain structures are allowed to form sequentially. Evidence for not only individual domain formation but also oligomerisation at a cotranslational stage has been shown in the cytosol and might occur in the ER as well. The drawback of cotranslational folding is that correct folding of a complete domain is still not immediately possible. The folding rate is faster than the insertion rate and the amount of misfolded intermediates must be limited. If the sequence of the domain is noncontiguous the situation is even more complicated. In multi-domain proteins, another obstacle is that once a domain is formed it is still fairly unstable due to the lack of interaction with the other domains. Moreover, the domain must still be prevented from interacting with the wrong parts of the protein during biosynthesis.

Another feature of in vivo folding is the dependency on folding chaperones and enzymes present in the ER. Both the potential problem with high protein content and the drawbacks of cotranslational insertion are overcome by the abundance and variety of different ER chaperones and enzymes. Moreover, the ER chaperones contribute greatly to the maturation of proteins during posttranslational modifications such as glycosylation, disulfide bond formation and proline and lysine hydroxylation. ER enzymes and chaperones make these posttranslational modifications possible and the modifications are in many cases not only necessary for the protein to be functional but also necessary for the protein to achieve its native structure. The chaperones have requirements themselves to perform efficiently and one prerequisite shared by many is a high Ca^{2+} concentration [31], which inside the ER is 10,000-fold higher than in the cytosol [32]. Moreover, enzymes involved in disulfide bond formation are able to function efficiently due to the highly oxidising environment in the ER [33]. The ratio of reduced and oxidised glutathione (GSH/GSSH) is 3:1 in the ER as compared to 100:1 in the cytosol, providing the ER with a redox environment favouring disulfide bond formation [34]. This provides an explanation for why recombinant disulfide bond containing proteins are generally not secreted from bacterial cells. Instead these proteins aggregate and accumulate in the cytosol. Only after special chemical treatment, these proteins may fold into their native conformation. On initial inspection the environment in the ER does not appear conductive for correct folding of a nascent polypeptide. However, thanks to the chaperones and reducing conditions, the ER is indeed an outstanding place for protein folding and maturation.

3. Chaperones

The folding and quality control of newly synthesised proteins in the ER is a sophisticated process involving several components. Four modes of action for the ER accessory molecules such as chaperones are described in Sections 3.1–3.4. Classical chaperone action is illustrated by BiP. The ER lectins calnexin and calreticulin are used as examples of substrate feature specific molecules. Folding catalysts are illustrated by looking at the families of peptidyl-prolyl isomerases and protein disulfide isomerases. Finally the functional group of dedicated chaperones are exemplified by looking at the MHC class I dedicated chaperone tapasin. Importantly, a single chaperone may act in more than one mode.

3.1. BiP

The group of classical ER chaperones has many members and includes several glucose-regulated proteins (GRPs). One of the most abundant classical molecular chaperones in the

ER is the immunoglobulin binding protein (BiP). BiP functions as a bona fide chaperone to stabilise newly synthesised polypeptides during folding, prevent aggregation, mediate retention in the ER and suppress formation of nonnative disulfide bonds [34]. In addition, BiP is also involved in the translocation of newly synthesised proteins across the ER membrane and in degradation of misfolded proteins. Classical chaperones bind to stretches of hydrophobic residues and the binding is dependent on the intrinsic ATPase activity of the chaperone. These chaperones are up-regulated and are of particular importance during conditions of cellular stress when misfolded proteins accumulate in the ER.

3.2. Calnexin and calreticulin

Some chaperones, such as the so-called lectins, have greater substrate specificity. Lectins are by definition non-enzymatic sugar-binding proteins (lectin-binding proteins). In the ER, they are represented by the unconventional chaperones calnexin and calreticulin. Calnexin and calreticulin interact specifically with monoglucosylated N-glycosylated proteins. N-linked glycosylation begins with addition of a large preformed oligosaccharide $Glc_3Man_9(GlcNAc)_2$ to an asparagine residue in the sequence Asn-X-Ser/Thr (X is any amino acid except for proline). However, not all possible glycosylation sites become glycosylated. $Glc_3Man_9(GlcNAc)_2$ is transferred to newly inserted polypeptides as they enter the ER and the oligosaccharide chain is further modified as the protein pass through the ER and Golgi apparatus. Calnexin and calreticulin are able to bind to the glycan after the high-mannose triglucose is trimmed to a monoglucose by glucosidase I and glucosidase II [25]. Once the substrate dissociates from calnexin/calreticulin, it is further trimmed and the third glucose is removed. However, if the glycoprotein is not correctly folded it will be recognised by UDP-glucose glucosyltransferase (UGGT) and will be re-monoglucosylated [25]. Again the ER lectins bind the reglucosylated $Glc_1Man_{7-9}(GlcNAc)_2$ oligosaccharides. Calnexin and calreticulin play an important role for the ER quality control of many glycoproteins through this cycle of de- and reglucosylation [35,36]. The processing of glycans in the ER is a basic mechanism in all cells and of immense significance for allowing many proteins to achieve their native structure. The longer time a protein spends in the calnexin–calreticulin cycle, the higher is the possibility that it achieves the correct folding. It has been suggested that all glycoproteins interact with ER lectins in mammalian cells [37]. Several reports have shown that prevention of N-linked glycosylation by either mutations in glycoprotein substrates or treatment with inhibitors such as tunicamycin results in misfolded proteins [38–42]. However, other studies have shown that glycosylation is dispensable for correct folding [43–45]. Although N-linked glycosylation is a definite requisite for correct folding of many glycoproteins, the effect of glycosylation is difficult to predict since it varies depending on the substrate.

Similar substrate-binding properties have been shown for calnexin and calreticulin in cell-free assays, but the in vivo substrate binding differs between the two. The topology, with calnexin being membrane-bound and calreticulin being soluble, has been suggested to be of significance for the binding preferences. In this model, the positions of glycan moieties in the substrate proteins direct interaction towards the most easily accessible lectin. Glycans located close to the membrane are more likely to interact with calnexin and glycans further away have a preference for interaction with the soluble lectin calreticulin. Studies with soluble calnexin and artificially membrane-bound calreticulin have shown support for this model [46,47].

Originally calnexin and calreticulin were suggested to interact only with monoglucosylated proteins but this view has been called into question since it has been shown that both calnexin and calreticulin can interact with non-glycosylated substrates [48–51]. Binding of calnexin and calreticulin to polypeptide stretches implies that they may function as bona fide ER chaperones in addition to their glycan dependent function. Williams and co-workers have shown that in cell-free assays, calreticulin and a soluble form of calnexin can function as classical molecular chaperones [49,50]. The lectins were shown to interact with glycoproteins devoid of monoglucose glycan moieties and also with non-glycosylated proteins, indicating a function distinct from that of a typical lectin. Interaction between calnexin and non-glycosylated proteins has also been detected in intact cells [52]. Moreover, in a microsome model both in vitro translated wild-type VSV glycoprotein and non-glycosylated mutant forms coprecipitated with calnexin [53]. The results from this study suggests that calnexin binding to non-glycosylated proteins is nonspecific and a consequence of aggregates formed by misfolded substrate proteins when N-linked glycosylation is prevented. A role for calnexin and calreticulin binding to polypeptide stretches and targeting misfolded proteins for proteasomal degradation has been proposed. Recently, both calnexin and calreticulin were shown to suppress aggregation of unfolded proteins via a polypeptide-binding site in their globular domains [54]. Misfolded α1-antitrypsin was shown to require calnexin binding for proteasomal degradation [55]. Furthermore, thermal aggregation assays showed that calreticulin forms high-molecular weight complexes with the human MHC class I allele HLA-A2 at 50 °C but not at 37 °C, indicating polypeptide-based interaction and a role of calreticulin in binding to misfolded proteins [56]. The dual function of calnexin and calreticulin in both productive folding, as lectins, and in binding to aggregates for disposal, as nonspecific chaperones, remains to be further elucidated.

3.3. Peptidyl isomerases and ERp57

Some proteins directly increase the rate of folding acting as true reaction catalysts (reviewed in Ref. [57]). One set of catalysing proteins present in the ER is the peptidyl-prolyl

isomerase family that facilitates the rotation around peptidyl-prolyl bonds of nascent polypeptides [58,59]. These enzymes catalyse the formation of a kinetically more favourable state of the peptide bond preceding proline residues. Most peptidyl-prolyl isomerases are able to catalyse the rotation of bonds of any exposed peptidyl-prolyl but examples of substrate specificity have been demonstrated (see Section 3.4 on dedicated accessory proteins).

The folding catalysts also include enzymes of the protein-disulfide isomerase (PDI) family responsible for the formation of disulfide bonds [34]. PDI, the archetype protein that also gives it name to the family, is also one of the most extensively studied members and acts on a wide range of disulfide bond containing proteins. PDI is one of the chaperones that are transcriptionally up-regulated in response to accumulation of unfolded proteins in the cell. This transcriptional up-regulation of a specific set of chaperones is called the unfolded protein response (UPR) [60].

ERp57 is another member of the PDI family and has two thiol/disulfide oxidoreductase active sites [61]. ERp57 was originally shown to interact specifically with N-glycosylated substrates [62–64], but no lectin-like properties were found in ERp57 [65]. It was therefore proposed and later proved that ERp57 interacts with ER lectins to assist in folding of N-glycosylated substrates [64,65]. ERp57 interacts specifically with both calnexin and calreticulin in both the presence and absence of glycoprotein substrates [66].

3.4. Tapasin and other dedicated accessory molecules

Finally, the dedicated proteins assist specific target molecules to achieve their correct final stable conformation and to be transported outside the ER. An early example was the product of the SH3 gene in yeast. The SH3 protein was found to specifically promote maturation of amino permeases but not other proteins [67]. One more recently discovered chaperone is the yeast PDI related protein, EPS1. EPS1 is suggested to function as a dedicated chaperone for the yeast plasma membrane ATPase PMA1 [68]. The list of dedicated chaperones in various organisms is rapidly growing (reviewed in Ref. [69]). However, the mechanisms for several of these chaperones, such as the mammalian procollagen folding factor HSP47 [70], remain to be elucidated. A well-known dedicated chaperone whose mechanism has been characterised in greater detail is the molecule receptor-associated protein (RAP). RAP escorts low density lipoprotein receptor-related protein (LRP) out of the ER to the Golgi [71]. During the transport, RAP prevents LRP aggregation but also stops LRP from interacting with ligands before it reaches the Golgi. RAP has also been found associated with other transmembrane receptors. Another protein with potential escort function is NinaA found in *Drosophila*. NinaA is a prolyl *cis–trans* isomerase and is required for proper maturation of two homologous rhodopsins [72]. NinaA is expressed in a cell type-specific manner and colocalises with opsin in the ER as well as in

secretory vesicles of the secretory pathway [72,73]. Mutation of NinaA leads to dramatic accumulation of opsins in the ER of photoreceptor cells [73]. NinaA is specifically needed for two forms of opsins, whereas a third more distantly related form is unaffected by NinaA mutations indicating the specificity of NinaA. MHC class II transport to the endosomes for peptide loading is also dependent on a specialised escort protein, known as the invariant chain (Ii) [74]. Another task for dedicated chaperones is the retention in the ER of maturing molecules by specific interactions, such as egasyn retention of β-glucoronidase [75] and carboxylesterase retention of C-reactive protein [76]. Several potential mediators of COP (coat protein)-coated vesicle transport have been identified including ERGIC-53 [77], the p24 family [78], tapasin [79] and BAP31 [80]. These receptors exhibit various degrees of substrate specificity. One outstanding example is tapasin that has a very high degree of specificity for its substrate. Tapasin interacts exclusively with MHC class I molecules and mediates recycling in COPI-coated vesicles of nonoptimally loaded class I molecules back to the ER [79]. The importance of the different receptors varies and some of them may act more as an all-purpose quality control than as an absolute requirement [81].

3.5. Chaperones work in concert

The independent action as well as the cooperation of chaperones are necessary for correct folding. Some chaperones bind immature proteins thereby forming a scaffold, allowing other chaperones to access the immature protein. Synergistic behaviour has been shown for BiP together with other chaperones. BiP cooperation with PDI has been shown for the folding of antibodies in vitro [82] and with calreticulin for the folding of coagulation factor VIII [83]. A kinetic protein-folding model has been proposed where BiP functions as a chaperone and PDI as a catalyst, and this model might also apply for other pairs of ER folding chaperones and catalysts [84]. The interplay between chaperones forms advanced networks and these are dependent on the state of the cell.

4. MHC class I maturation

MHC class I molecules are trimeric complexes dependent on proper and assisted assembly. HC and β2-m assemble in the ER and are loaded with peptide before export to the cell surface. Assembly of the HC–β2-m dimer has been suggested to take place cotranslationally and precede peptide binding [85]. The proteasome-generated peptides are transported from the cytosol into the ER by the transporter associated with antigen processing (TAP) [86]. TAP preferentially transports peptides 8–12 amino acids in length although peptides as long as 40 amino acids can be transported [87]. MHC class I molecules present peptides of

usually eight or nine amino acids in length [88], suggesting a role for ER proteases in trimming of TAP transported peptides to generate suitable MHC class I ligands. Trimming of peptides in the ER by aminopeptidases has been shown and it has even been suggested that trimming may occur even after peptide loading onto MHC class I [89]. Recently, the aminopeptidase ERAAP was described to be essential for the production of certain MHC class I ligands [90,91]. Optimal peptides are stably loaded onto class I molecules through interactions with specific anchor pockets in the class I peptide binding groove. The binding is further stabilised by binding energy from the C- and N-termini of the peptide and of the peptide backbone. The complementary nature of the binding groove and the peptide ligand has been demonstrated by biochemical peptide elution experiments [92,93] and by solving the structure of different class I–peptide complexes by X-ray crystallographic studies [94–96].

The early stage of MHC class I maturation includes HC binding to the chaperones BiP and calnexin. When HLA-HC binds β2-m, calnexin is replaced by calreticulin whereas the situation for mouse MHC class I alleles (H2) HC seems to be somewhat different (as described in Section 4.2). The HC–β2-m dimer subsequently forms part of the MHC class I peptide loading complex (LC) containing, in addition, tapasin, calreticulin, ERp57, TAP and perhaps also other proteins [97]. Fig. 1 illustrates the cooperation between accessory proteins in the ER quality control. Here the cooperativity concept is specifically illustrated for MHC class I maturation showing the teamwork of chaperones, TAP and ERAAP.

A large proportion of nascent HC polypeptides never folds successfully but is instead degraded at an early stage [98]. The proportion of properly folded and expressed MHC class I molecules is allele-dependent [99]. Moreover, the intrinsic stability, the dependence on glycosylation, and chaperone interaction and the quality control steps all differ for products of different class I alleles [20,99–101].

4.1. Roles of BiP and calnexin in early stage maturation of MHC class I folding

During synthesis on the ribosomes, MHC class I HCs are cotranslationally inserted into the ER through the translocon. The signal peptide is cleaved off at once and ER chaperones associate with the growing HC [17,102–105]. Human HC has been shown to associate with both BiP and calnexin during early stage maturation. BiP binds human HC at a stage before or simultaneously with calnexin and a sequential mode of interaction has been proposed [102,106]. Association of HLA-HC and BiP has been shown with biochemical methods in β2-m-deficient cells where the maturation is arrested at an early stage. However, association of BiP with class I molecules in mouse has so far not been detected and could result from the differences in the murine and human β2-m-deficient cell lines available or possibly from differences in glycosylation between H2- and HLA-HCs (see Section 4.2). Both BiP and calnexin bind to the ER translocation machinery. BiP binds the ER translocon and calnexin binds directly to the ribosome through its C-terminal cytoplasmic tail [107]. The early chaperones
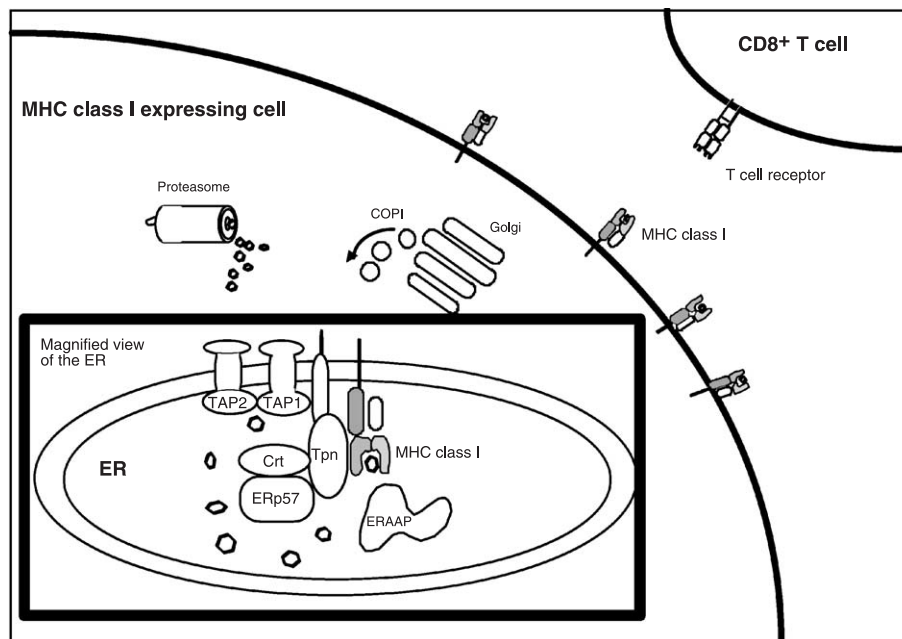

Fig. 1. Schematic picture of antigen presentation in the context of MHC class I molecules. Peptides generated by proteasomes are transported through the ER membrane by TAP. Accessory proteins cooperate to allow MHC class I molecules to be loaded with optimal peptide and be presented at the cell surface. At the cell surface CD8+ T cells scan the typically 10,000 MHC class I molecules expressed at each cell. Abbreviations: HC—heavy chain, β2-m—β2-microglobulin, cnx—calnexin, crt—calreticulin, tpn—tapasin, ERAAP—ER associated aminopeptidase.

mediate polypeptide folding during vectorial translocation as well as preventing premature degradation of the immature proteins. Stretches of hydrophobic amino acids suitable for BiP binding occur in proteins, on average every 40 residues, allowing BiP to interact cotranslationally with the majority of nascent polypeptides. Calnexin binding, on the other hand, occurs first when an N-linked glycosylation site has been properly inserted, glycan has been added and subsequently monoglucosylated [108,109].

4.2. Topology of the ER lectins differs and determines their substrate interaction patterns

Differences in MHC class I glycosylation sites together with the different topology of calnexin and calreticulin are likely to account for differences in mouse and human HC chaperone association. Human class I HC has a single N-linked glycosylation sequon (Asn-X-Ser/Thr, X is any amino acid except for proline) at Asparagine 86 (Asn[86]) while mouse HCs in addition have either one or two more sequons [110]. Since the HC is inserted into the ER N-terminal first the glycosylation sequon is initially located close to the ER membrane, and calnexin, the membrane-bound lectin, easily gains access to the Asn[86] glycan. The conformation change of the HC when β2-m binds either allows or requires the soluble calreticulin to replace calnexin.

In mouse the situation is to some extent different since calnexin remains associated with HC after β2-m binds. The proposal that the prolonged association of calnexin with mouse HC, in comparison to human HC, results from differences in the number and location of glycosylation sequons has been supported by several different experimental systems. Mouse HC expressed in human cells preserves the chaperone interaction pattern typical for H2 [102]. Moreover, studies of mutant human HC with a second glycan introduced reveal an assembly process similar to mouse HC with respect to lectin association [111].

The topology theory has been strongly supported and one study has shown that calreticulin can be replaced by soluble calnexin for HLA maturation [47]. The initial interaction of HC with calnexin is also thought to be facilitated by the direct interaction of calnexin with the polypeptide synthesising ribosome [112]. Recently, both calnexin and calreticulin have been shown to exhibit classical molecular chaperone activity when interacting with non-glycosylated proteins in vitro [49,50]. Inhibition of the glucosidases responsible for removing the glucoses from the core oligosaccharide by castanospermine abolished the interaction of calreticulin with class I molecules [20], indicating the bona fide action to be secondary to the lectin-like activity. However, non-glycosylated MHC class I molecules have been demonstrated to bind calnexin, obviously via a non-lectin-like activity [52]. These calnexin bound class I molecules were in an open conformation and accumulated in the ER indicative of nonproductive folding and a role of nonspecific calnexin binding to misfolded class I. ATP

depletion in β2-m-deficient cells resulted in accumulation of GFP-tagged H2K[b] as well as calnexin at ER subregions distinct from the ER-exit sites used by assembled class I [113]. Interestingly, no accumulation of class I at any ER region was found in the presence of ATP. Instead these molecules had very high mobility. The precise involvement of calnexin in quality control and its possible role in the degradation of misfolded class I remain to be elucidated. The bona fide action of ER lectins concerning protein maturation in general and specifically MHC class I maturation remains to be further investigated.

4.3. ER lectins are a prerequisite for proper MHC class I maturation

The importance of calnexin and calreticulin in the ER quality control is obvious since the longer time a glycoprotein spends in the calnexin–calreticulin binding cycle, the more likely that accurate folding of the protein will be achieved. However, glycoproteins differ in their dependence on glycosylation for correct folding, which is exemplified by many well-studied viral proteins such as the VSV-G protein [10]. Different strains of VSV show different dependence on glycosylation for G protein maturation [10,11].

Carreno et al. [52] showed that MHC class I molecules deficient in glycosylation accumulate in the ER in an open conformation. However, both unglycosylated as well as fully glycosylated HCs were able to interact with calnexin in this study. This might indicate a requirement for glycosylation as an admission ticket to the calnexin–calreticulin quality control cycle while HC–calnexin association during nonproductive folding is independent of the glycosylation state. Studies of MHC class I maturation in human cells where calnexin association has been abolished have given different results. Defects in maturation of HLA as well as in VSV G protein have been shown when binding to calnexin is hindered. Furthermore, mouse MHC class I molecules were shown to misfold and aggregate in the absence of calnexin [16]. However, another study showed that assembly and transport of various H2 alleles transfected into the human calnexin-deficient cell line CMN.NK[R] are not significantly impaired in the absence of calnexin [114]. Other studies of MHC class I assembly in human cells showed normal class I assembly regardless of the presence of calnexin [114,115]. The discrepancy might be due to different models used and species differences in class I maturation. The maturation rate of different class I alleles is remarkably different [116,117] and might further reflect the dynamic interplay between substrate and ER chaperones. In human cells the simultaneous interaction of BiP and calnexin with class I HC has been suggested to explain the unaffected maturation of MHC class I in the absence of calnexin [102]. Also, overlapping function of calnexin and calreticulin has been suggested to allow proper MHC class I folding in human calnexin-depleted cells [115,118]. The overlapping substrate range

for classical chaperones and the ER lectins is well illustrated at this stage of class I maturation.

Mouse class I molecules have also been shown to be chaperoned by calnexin during late stage association with tapasin and TAP, and it was originally proposed that calreticulin was redundant in this system. However, recent experiments in calreticulin knock-out mice have shown that absence of calreticulin results in impaired peptide loading and low stability of class I [119]. Importantly, calreticulin deficiency did not directly impair incorporation of class I into the MHC class I peptide loading complex. Transfecting the cells with calreticulin cDNA but not with soluble calnexin cDNA restored the MHC class I surface expression. This suggests a specific requirement for calreticulin for a functional loading complex, which cannot be replaced by a homologous lectin binding protein. The essential function of calreticulin for class I expression might indicate the need for a structural scaffold on which other loading complex components (e.g. ERp57 and tapasin) are dependent for efficient binding to class I molecules. In this complex calreticulin may be initially recruited by monoglycans but the binding might be sustained independently of its lectin-like properties.

4.4. ERp57 interacts with distinct folding intermediates of MHC class I

The best characterised function of ERp57 is its thiol-dependent reductase activity, which catalyses the formation of protein disulfide bonds [120,121]. A role for ERp57 in MHC class I assembly has been demonstrated [18,19,122]. Two disulfide bonds are formed in the class I HC, the first in the α-3 domain and the second in the peptide binding α-2 domain. The importance of correctly formed disulfide bonds is illustrated by mutation analyses in either of these bonds, which leads to misfolding of class I in the ER and results in deficient cell surface expression [123–125].

Whether there is an interaction or not between free HC and ERp57 has been a question of controversy but independent reports have now shown that ERp57 associates with calnexin-bound HC at a stage prior to HC association with the loading complex [19,126]. Formation of the first disulfide bond of the HC, located in the α-3 domain, takes place soon after calnexin has bound and corresponds temporally to the association of HC–calnexin with ERp57 [19,127]. It is also possible that the second disulfide bond is formed at this stage as has been shown for HLA-B27 [126]. ERp57 is not able to bind HC in the human calnexin-deficient cell line CEM.NKR but is instead proposed to be replaced by another ER chaperone, ERp72 [128]. MHC class I maturation has not been found to be impaired in this cell line.

ERp57 is also found associated with MHC class I in the loading complex [97,128]. This might indicate that not all TAP-associated MHC class I molecules have correctly formed disulfide bonds. The most intriguing explanation for this is that some class I molecules remain partially folded, or that the α-2 domain bond is formed and broken and reformed, allowing exchange of suboptimal peptides with optimal ones. When high-affinity peptide is loaded, this will trigger stable formation of the second disulfide bond and finally export of the stable class I molecule. Indeed, conformational changes in class I molecules have been observed after peptide binding [124,129]. Possibly these changes might be due to finalising aminopeptidase peptide trimming by ERAAP. Disulfide bonded tapasin-ERp57 has been detected in the loading complex and, interestingly, mutation of cysteine 95 in tapasin not only prevents disulfide bond formation of the ERp57–tapasin complex but also results in incompletely oxidised MHC class I molecules [130]. Class I molecules in this study exited the ER in an unstable conformation. Further studies need to elucidate the exact role of ERp57 and partially oxidized MHC class I in the loading complex.

4.5. Tapasin is an MHC class I dedicated chaperone

When the class I HC–β2-m dimer is formed, it binds peptide to finalise the conformation. If the peptide fits the binding groove well, the class I molecule is stable and will be transported to the cell surface. On the other hand, if the class I molecule receives a suboptimal peptide, further action of ER chaperones is required. The MHC class I molecule is then incorporated into the loading complex by binding to tapasin [21,131]. Tapasin is a 48-kDa glycoprotein and is stably bound to TAP. Tapasin not only bridges class I to the loading complex but also influences the efficiency of peptide binding to TAP in the cytosol [132]. We found that tapasin binds to the β- and γ-subunits of COPI-coated vesicles [79]. Moreover, MHC class I molecules were found to be COPI-associated in the presence of tapasin but not in its absence. Subcellular fractionation revealed that the COPI-associated MHC class I molecules were found as late as in the Golgi network and that they were peptide-receptive. A model where tapasin mediates recycling of suboptimally loaded class I molecules to the ER from late secretory compartments is suggested. In this way, unstable class I molecules are allowed a second chance to receive stable native conformation. Tapasin knock-out mice have reduced cell surface expression of MHC class I and are deficient in eliciting T cell-mediated immune responses [133,134]. Tapasin is clearly essential for the maturation of MHC class I molecules and represents one member of a possibly very large group of dedicated chaperones of the ER.

4.6. The loading complex is a highly cooperative unit with multiple interactions between the components

The high extent of cooperativity between ER chaperones is well illustrated by examining the organisation of the MHC class I loading complex. Recently the binding of

calreticulin to class I was shown to be independent of tapasin [135,136]. Other studies have demonstrated that tapasin is a prerequisite for calreticulin binding to TAP associated class I [18]. Tapasin is vital for proper MHC class I cell surface expression as well as for the formation of the loading complex [21,131]. Studies of the mutant B cell line 721.220, which is deficient in tapasin, show reduced amounts of both calreticulin and ERp57 in the loading complex [18,137]. Class I molecules are not found associated with TAP in these cells [100]. As a consequence, the amount of class I associated with both ERp57 and calreticulin is reduced in this cell line [18,20]. Tapasin forms disulfide bond-linked intermediates with ERp57 and mutagenesis of tapasin cysteine residue 95 abolishes this interaction [130]. In 721.220 cells transfected with tapasin Cys^{95} cDNA, the peptide loading onto MHC class I is suboptimal and unstable class I molecules are expressed at the cell surface. Together these data imply a complex structure with different kinds of interactions between the components of the loading complex. Multiple interactions between components of the loading complex clearly exist but the exact organisation and the nature of these interactions remains to be analysed in more detail. Moreover, the multiple chaperone interactions occurring both before and after HC association with the loading complex need to be further studied. So far, a partly sequential scheme has been established for the MHC class I maturation as illustrated in Fig. 2.

5. Conclusions

Achieving information about the maturation of multidomain proteins such as MHC class I is of great importance to understand the general principles of folding in the ER as well as revealing the biological functions of processed proteins. It is of significance to determine the relation between factors influencing folding in vitro and in vivo. Moreover, principles of folding are related to the effects of molecular crowding. Both folding kinetics and thermodynamics need to be studied in the highly crowded environment of the ER to be able to proceed in the area of in vitro folding. Also it is necessary to distinguish between the general crowding effect on newly synthesised polypeptides which result in assembly of multi component complexes, enhancement of polypeptide chains into native structures and the specific effects of crowding which increase the efficiency of chaperones and dedicated molecules, in turn promoting correct folding.

The abundance and redundancy of chaperones raise folding accuracy significantly. The multiple chaperones present result in a high success rate in protein maturation of newly synthesised proteins even under conditions of cellular stress and the UPR further points to the importance of chaperones. The discovery of dedicated accessory molecules provides totally new insights into the ER quality control and its diversity of mechanisms. The discovery of several specialised chaperones such as HSP47, RAP and tapasin will most likely be followed by discoveries of a wide array of dedicated proteins helping in the maturation of specific substrate proteins.

The urge to study and elucidate chaperone action in the ER is highlighted by the role of chaperones in multiple diseases. Aggregation of proteins is a phenomenon in a growing number of diseases such as Alzheimer's, Creutzfeldt-Jacob, transmissible spongiform encephalopathies and cystic fibrosis [138]. Moreover, chaperone-assisted MHC
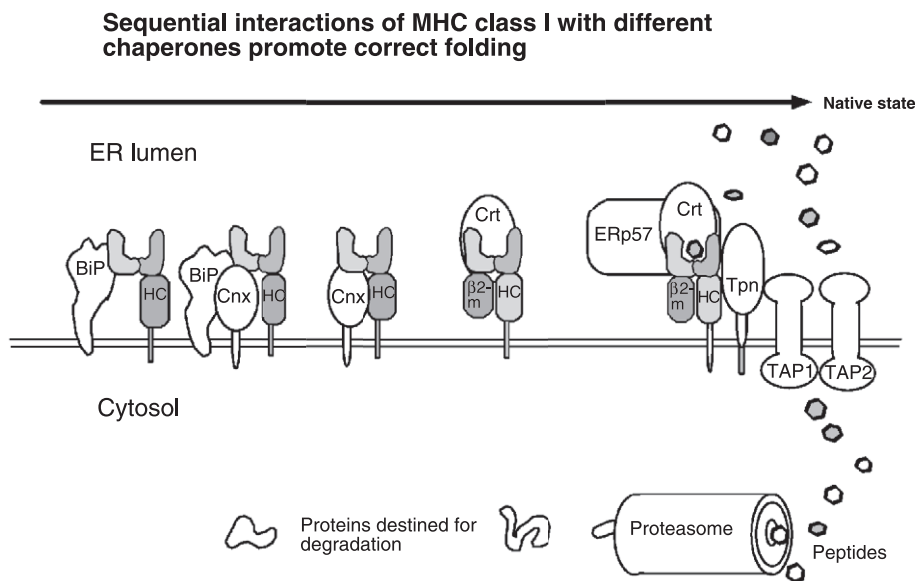

Fig. 2. MHC class I molecules assemble in the ER to stable native trimeric complexes consisting of heavy chain, β2-microglobulin and peptide. Different chaperones cooperate in a sequential mode to control the quality of the maturing molecule. Abbreviations: HC—heavy chain, β2-m—β2-microglobulin, cnx—calnexin, crt—calreticulin, tpn—tapasin.

class I maturation has been shown to be specifically targeted by different viral inhibitory strategies and the variety of these viral mechanisms has been proven to be substantial [139].

Acknowledgements

References

[1] J. Klein, Biology of the Mouse Histocompatibility-2 Complex, Springer, New York, 1975.

[2] A.R. Townsend, J. Rothbard, F.M. Gotch, G. Bahadur, D. Wraith, A.J. McMichael, The epitopes of influenza nucleoprotein recognized by cytotoxic T lymphocytes can be defined with short synthetic peptides, Cell 44 (1986) 959–968.

[3] K.L. Rock, A.L. Goldberg, Degradation of cell proteins and the generation of MHC class I-presented peptides, Annu. Rev. Immunol. 17 (1999) 739–779.

[4] M. Gromme, J. Neefjes, Antigen degradation or presentation by MHC class I molecules via classical and non-classical pathways, Mol. Immunol. 39 (2002) 181.

[5] R.M. Zinkernagel, P.C. Doherty, Restriction of in vitro T cell-mediated cytotoxicity in lymphocytic choriomeningitis within a syngeneic or semiallogeneic system, Nature 248 (1974) 701–702.

[6] P.J. Bjorkman, M.A. Saper, B. Samraoui, W.S. Bennett, J.L. Strominger, D.C. Wiley, The foreign antigen binding site and T cell recognition regions of class I histocompatibility antigens, Nature 329 (1987) 512–518.

[7] A. Townsend, C. Ohlen, J. Bastin, H.G. Ljunggren, L. Foster, K. Karre, Association of class I major histocompatibility heavy and light chains induced by viral peptides, Nature 340 (1989) 443–448.

[8] A. Townsend, T. Elliott, V. Cerundolo, L. Foster, B. Barber, A. Tse, Assembly of MHC class I molecules analyzed in vitro, Cell 62 (1990) 285–295.

[9] R. Gibson, S. Kornfeld, S. Schlesinger, The effect of oligosaccharide chains of different sizes on the maturation and physical properties of the G protein of vesicular stomatitis virus, J. Biol. Chem. 256 (1981) 456–462.

[10] C. Hammond, A. Helenius, Folding of VSV G protein: sequential interaction with BiP and calnexin, Science 266 (1994) 456–458.

[11] M.E. Mathieu, P.R. Grigera, A. Helenius, R.R. Wagner, Folding, unfolding, and refolding of the vesicular stomatitis virus glycoprotein, Biochemistry 35 (1996) 4084–4093.

[12] M. Molinari, A. Helenius, Glycoproteins form mixed disulphides with oxidoreductases during folding in living cells, Nature 402 (1999) 90–93.

[13] W. Chen, A. Helenius, Role of ribosome and translocon complex during folding of influenza hemagglutinin in the endoplasmic reticulum of living cells, Mol. Biol. Cell 11 (2000) 765–772.

[14] D.B. Williams, B.H. Barber, R.A. Flavell, H. Allen, Role of beta 2-microglobulin in the intracellular transport and surface expression of murine class I histocompatibility molecules, J. Immunol. 142 (1989) 2796–2806.

[15] Q. Zhang, M. Tector, R.D. Salter, Calnexin recognizes carbohydrate and protein determinants of class I major histocompatibility complex molecules, J. Biol. Chem. 270 (1995) 3944–3948.

[16] A. Vassilakos, M.F. Cohen-Doyle, P.A. Peterson, M.R. Jackson, D.B. Williams, The molecular chaperone calnexin facilitates folding and assembly of class I histocompatibility molecules, EMBO J. 15 (1996) 1495–1506.

[17] K.M. Paulsson, P.O. Anderson, S. Chen, H.O. Sjogren, H.G. Ljunggren, P. Wang, S. Li, Assembly of tapasin-associated MHC class I in the absence of the transporter associated with antigen processing (TAP), Int. Immunol. 13 2001, 23–29.

[18] E.A. Hughes, P. Cresswell, The thiol oxidoreductase ERp57 is a component of the MHC class I peptide-loading complex, Curr. Biol. 8 (1998) 709–712.

[19] J.A. Lindquist, O.N. Jensen, M. Mann, G.J. Hammerling, ER-60, a chaperone with thiol-dependent reductase activity involved in MHC class I assembly, EMBO J. 17 (1998) 2186–2195.

[20] B. Sadasivan, P.J. Lehner, B. Ortmann, T. Spies, P. Cresswell, Roles for calreticulin and a novel glycoprotein, tapasin, in the interaction of MHC class I molecules with TAP, Immunity 5 (1996) 103–114.

[21] S. Li, H.O. Sjogren, U. Hellman, R.F. Pettersson, P. Wang, Cloning and functional characterization of a subunit of the transporter associated with antigen processing, Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 8708–8713.

[22] C.M. Dobson, M. Karplus, The fundamentals of protein folding: bringing together theory and experiment, Curr. Opin. Struct. Biol. 9 (1999) 92–101.

[23] B. van den Berg, R.J. Ellis, C.M. Dobson, Effects of macromolecular crowding on protein folding and aggregation, EMBO J. 18 (1999) 6927–6933.

[24] E.S. Trombetta, A. Helenius, Lectins as chaperones in glycoprotein folding, Curr. Opin. Struct. Biol. 8 (1998) 587–592.

[25] A.J. Parodi, Protein glucosylation and its role in protein folding, Annu. Rev. Biochem. 69 (2000) 69–93.

[26] J. Frydman, Folding of newly translated proteins in vivo: the role of molecular chaperones, Annu. Rev. Biochem. 70 (2001) 603–647.

[27] S.B. Zimmerman, A.P. Minton, Macromolecular crowding: biochemical, biophysical, and physiological consequences, Annu. Rev. Biophys. Biomol. Struct. 22 (1993) 27–65.

[28] A.B. Fulton, How crowded is the cytoplasm? Cell 30 (1982) 345–347.

[29] A.P. Minton, The effect of volume occupancy upon the thermodynamic activity of proteins: some biochemical consequences, Mol. Cell. Biochem. 55 (1983) 119–140.

[30] S.B. Zimmerman, S.O. Trach, Estimation of macromolecule concentrations and excluded volume effects for the cytoplasm of *Escherichia coli*, J. Mol. Biol. 222 (1991) 599–620.

[31] J. Meldolesi, T. Pozzan, The endoplasmic reticulum Ca2+ store: a view from the lumen, Trends Biochem. Sci. 23 (1998) 10–14.

[32] R. Chapman, C. Sidrauski, P. Walter, Intracellular signaling from the endoplasmic reticulum to the nucleus, Annu. Rev. Cell Dev. Biol. 14 (1998) 459–485.

[33] C. Hwang, A.J. Sinskey, H.F. Lodish, Oxidized redox state of glutathione in the endoplasmic reticulum, Science 257 (1992) 1496–1502.

[34] M.J. Gething, J. Sambrook, Protein folding in the cell, Nature 355 (1992) 33–45.

[35] C. Hammond, I. Braakman, A. Helenius, Role of N-linked oligosaccharide recognition, glucose trimming, and calnexin in glycoprotein folding and quality control, Proc. Natl. Acad. Sci. U. S. A. 91 (1994) 913–917.

[36] J.R. Peterson, A. Ora, P.N. Van, A. Helenius, Transient, lectin-like association of calreticulin with folding intermediates of cellular and viral glycoproteins, Mol. Biol. Cell 6 (1995) 1173–1184.

[37] A.J. Parodi, Role of N-oligosaccharide endoplasmic reticulum processing reactions in glycoprotein folding and degradation, Biochem. J. 348 (1) 2000, 1–13.

[38] A.K. Taylor, R. Wall, Selective removal of alpha heavy-chain glycosylation sites causes immunoglobulin A degradation and reduced secretion, Mol. Cell. Biol. 8 (1988) 4197–4203.

[39] S. Dube, J.W. Fisher, J.S. Powell, Glycosylation at specific sites of erythropoietin is essential for biosynthesis, secretion, and biological function, J. Biol. Chem. 263 (1988) 17516–17521.

[40] G.V. Ronnett, V.P. Knutson, R.A. Kohanski, T.L. Simpson, M.D. Lane, Role of glycosylation in the processing of newly translated insulin proreceptor in 3T3-L1 adipocytes, J. Biol. Chem. 259 (1984) 4566–4575.

[41] L.J. Slieker, T.M. Martensen, M.D. Lane, Synthesis of epidermal growth factor receptor in human A431 cells. Glycosylation-dependent acquisition of ligand binding activity occurs post-translationally in the endoplasmic reticulum, J. Biol. Chem. 261 (1986) 15233–15241.

[42] R. Konig, G. Ashwell, J.A. Hanover, Glycosylation of CD4. Tunicamycin inhibits surface expression, J. Biol. Chem. 263 (1988) 9502–9507.

[43] P.P. Breitfeld, D. Rup, A.L. Schwartz, Influence of the N-linked oligosaccharides on the biosynthesis, intracellular routing, and function of the human asialoglycoprotein receptor, J. Biol. Chem. 259 (1984) 10414–10421.

[44] S.T. George, A.E. Ruoho, C.C. Malbon, N-glycosylation in expression and function of beta-adrenergic receptors, J. Biol. Chem. 261 (1986) 16559–16564.

[45] C.J. van Koppen, N.M. Nathanson, Site-directed mutagenesis of the m2 muscarinic acetylcholine receptor. Analysis of the role of N-glycosylation in receptor expression and function, J. Biol. Chem. 265 (1990) 20887–20892.

[46] I. Wada, S. Imai, M. Kai, F. Sakane, H. Kanoh, Chaperone function of calreticulin when expressed in the endoplasmic reticulum as the membrane-anchored and soluble forms, J. Biol. Chem. 270 (1995) 20298–20304.

[47] U.G. Danilczyk, M.F. Cohen-Doyle, D.B. Williams, Functional relationship between calreticulin, calnexin, and the endoplasmic reticulum luminal domain of calnexin, J. Biol. Chem. 275 (2000) 13089–13097.

[48] M. Jannatipour, M. Callejo, A.J. Parodi, J. Armstrong, L.A. Rokeach, Calnexin and BiP interact with acid phosphatase independently of glucose trimming and reglucosylation in *Schizosaccharomyces pombe*, Biochemistry 37 (1998) 17253–17261.

[49] Y. Ihara, M.F. Cohen-Doyle, Y. Saito, D.B. Williams, Calnexin discriminates between protein conformational states and functions as a molecular chaperone in vitro, Mol. Cell 4 (1999) 331–341.

[50] Y. Saito, Y. Ihara, M.R. Leach, M.F. Cohen-Doyle, D.B. Williams, Calreticulin functions in vitro as a molecular chaperone for both glycosylated and non-glycosylated proteins, EMBO J. 18 (1999) 6718–6729.

[51] C.S. Jorgensen, N.H. Heegaard, A. Holm, P. Hojrup, G. Houen, Polypeptide binding properties of the chaperone calreticulin, Eur. J. Biochem. 267 (2000) 2945–2954.

[52] B.M. Carreno, K.L. Schreiber, D.J. McKean, I. Stroynowski, T.H. Hansen, Aglycosylated and phosphatidylinositol-anchored MHC class I molecules are associated with calnexin. Evidence implicating the class I-connecting peptide segment in calnexin association, J. Immunol. 154 (1995) 5173–5180.

[53] K.S. Cannon, D.N. Hebert, A. Helenius, Glycan-dependent and -independent association of vesicular stomatitis virus G protein with calnexin, J. Biol. Chem. 271 (1996) 14280–14284.

[54] M.R. Leach, M.F. Cohen-Doyle, D.Y. Thomas, D.B. Williams, Localization of the lectin, ERp57-binding, and polypeptide-binding sites of calnexin and calreticulin, J. Biol. Chem. 6 (2002) 6.

[55] Y. Liu, P. Choudhury, C.M. Cabral, R.N. Sifers, Oligosaccharide modification in the early secretory pathway directs the selection of a misfolded glycoprotein for degradation by the proteasome, J. Biol. Chem. 274 (1999) 5861–5867.

[56] L. Mancino, S.M. Rizvi, P.E. Lapinski, M. Raghavan, Calreticulin recognizes misfolded HLA-A2 heavy chains, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 5931–5936.

[57] C. Schiene, G. Fischer, Enzymes that catalyse the restructuring of proteins, Curr. Opin. Struct. Biol. 10 (2000) 40–45.

[58] K. Lang, F.X. Schmid, G. Fischer, Catalysis of protein folding by prolyl isomerase, Nature 329 (1987) 268–270.

[59] S. Bose, R.B. Freedman, Peptidyl prolyl *cis–trans*-isomerase activity associated with the lumen of the endoplasmic reticulum, Biochem. J. 300 (1994) 865–870.

[60] K. Mori, Tripartite management of unfolded proteins in the endoplasmic reticulum, Cell 101 (2000) 451–454.

[61] D.M. Ferrari, H.D. Soling, The protein disulphide-isomerase family: unravelling a string of folds, Biochem. J. 339 (1999) 1–10.

[62] J.D. Oliver, R.C. Hresko, M. Mueckler, S. High, The glut 1 glucose transporter interacts with calnexin and calreticulin, J. Biol. Chem. 271 (1996) 13691–13696.

[63] J.G. Elliott, J.D. Oliver, S. High, The thiol-dependent reductase ERp57 interacts specifically with N-glycosylated integral membrane proteins, J. Biol. Chem. 272 (1997) 13849–13855.

[64] J.D. Oliver, F.J. van der Wal, N.J. Bulleid, S. High, Interaction of the thiol-dependent reductase ERp57 with nascent glycoproteins, Science 275 (1997) 86–88.

[65] A. Zapun, N.J. Darby, D.C. Tessier, M. Michalak, J.J. Bergeron, D.Y. Thomas, Enhanced catalysis of ribonuclease B folding by the interaction of calnexin or calreticulin with ERp57, J. Biol. Chem. 273 (1998) 6009–6012.

[66] J.D. Oliver, H.L. Roderick, D.H. Llewellyn, S. High, ERp57 functions as a subunit of specific complexes formed with the ER lectins calreticulin and calnexin, Mol. Biol. Cell 10 (1999) 2573–2582.

[67] P.O. Ljungdahl, C.J. Gimeno, C.A. Styles, G.R. Fink, SHR3: a novel component of the secretory pathway specifically required for localization of amino acid permeases in yeast, Cell 71 (1992) 463–478.

[68] Q. Wang, A. Chang, Eps1, a novel PDI-related protein involved in ER quality control in yeast, EMBO J. 18 (1999) 5972–5982.

[69] L. Ellgaard, M. Molinari, A. Helenius, Setting the standards: quality control in the secretory pathway, Science 286 (1999) 1882–1888.

[70] K. Nagata, Hsp47: a collagen-specific molecular chaperone, Trends Biochem. Sci. 21 (1996) 22–26.

[71] G. Bu, H.J. Geuze, G.J. Strous, A.L. Schwartz, 39 kDa receptor-associated protein is an ER resident protein and molecular chaperone for LDL receptor-related protein, EMBO J. 14 (1995) 2269–2280.

[72] M.A. Stamnes, B.H. Shieh, L. Chuman, G.L. Harris, C.S. Zuker, The cyclophilin homolog ninaA is a tissue-specific integral membrane protein required for the proper synthesis of a subset of *Drosophila* rhodopsins, Cell 65 (1991) 219–227.

[73] N.J. Colley, E.K. Baker, M.A. Stamnes, C.S. Zuker, The cyclophilin homolog ninaA is required in the secretory pathway, Cell 67 (1991) 255–263.

[74] P. Cresswell, Assembly, transport, and function of MHC class II molecules, Annu. Rev. Immunol. 12 (1994) 259–293.

[75] L. Zhen, M.E. Rusiniak, R.T. Swank, The beta-glucuronidase propeptide contains a serpin-related octamer necessary for complex formation with egasyn esterase and for retention within the endoplasmic reticulum, J. Biol. Chem. 270 (1995) 11912–11920.

[76] S. Macintyre, D. Samols, P. Dailey, Two carboxylesterases bind C-reactive protein within the endoplasmic reticulum and regulate its secretion during the acute phase response, J. Biol. Chem. 269 (1994) 24496–24503.

[77] C. Appenzeller, H. Andersson, F. Kappeler, H.P. Hauri, The lectin ERGIC-53 is a cargo transport receptor for glycoproteins, Nat. Cell Biol. 1 (1999) 330–334.

[78] M. Marzioch, D.C. Henthorn, J.M. Herrmann, R. Wilson, D.Y. Thomas, J.J. Bergeron, R.C. Solari, A. Rowley, Erp1p and Erp2p, partners for Emp24p and Erv25p in a yeast p24 complex, Mol. Biol. Cell 10 (1999) 1923–1938.

[79] K.M. Paulsson, M.J. Kleijmeer, J. Griffith, M. Jevon, S. Chen, P.O.

Anderson, H.O. Sjogren, S. Li, P. Wang, Association of tapasin and COPI provides a new mechanism for the retrograde transport of MHC class I molecules from the Golgi complex to the ER, J. Biol. Chem. 7 (2002) 7.

[80] W.G. Annaert, B. Becker, U. Kistner, M. Reth, R. Jahn, Export of cellubrevin from the endoplasmic reticulum is controlled by BAP31, J. Cell Biol. 139 (1997) 1397–1410.

[81] S. Springer, E. Chen, R. Duden, M. Marzioch, A. Rowley, S. Hamamoto, S. Merchant, R. Schekman, The p24 proteins are not essential for vesicular transport in Saccharomyces cerevisiae, Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 4034–4039.

[82] M. Mayer, U. Kies, R. Kammermeier, J. Buchner, BiP and PDI cooperate in the oxidative folding of antibodies in vitro, J. Biol. Chem. 275 (2000) 29421–29425.

[83] S.W. Pipe, J.A. Morris, J. Shah, R.J. Kaufman, Differential interaction of coagulation factor VIII and factor V with protein chaperones calnexin and calreticulin, J. Biol. Chem. 273 (1998) 8537–8544.

[84] R. Gonz lez, B.A. Andrews, J.A. Asenjo, Kinetic model of BiP- and PDI-mediated protein folding and assembly, J. Theor. Biol. 214 (2002) 529–537.

[85] J.J. Neefjes, G.J. Hammerling, F. Momburg, Folding and assembly of major histocompatibility complex class I heterodimers in the endoplasmic reticulum of intact cells precedes the binding of peptide, J. Exp. Med. 178 (1993) 1971–1980.

[86] M.T. Heemels, H. Ploegh, Generation, translocation, and presentation of MHC class I-restricted peptides, Annu. Rev. Biochem. 64 (1995) 463–491.

[87] B. Lankat-Buttgereit, R. Tampe, The transporter associated with antigen processing: function and implications in human diseases, Physiol. Rev. 82 (2002) 187–204.

[88] K. Falk, O. Rotzschke, H.G. Rammensee, Cellular peptide composition governed by major histocompatibility complex class I molecules, Nature 348 (1990) 248–251.

[89] N. Brouwenstijn, T. Serwold, N. Shastri, MHC class I molecules can direct proteolytic cleavage of antigenic precursors in the endoplasmic reticulum, Immunity 15 (2001) 95–104.

[90] T. Serwold, S. Gaw, N. Shastri, ER aminopeptidases generate a unique pool of peptides for MHC class I molecules, Nat. Immunol. 2 (2001) 644–651.

[91] T. Serwold, F. Gonzalez, J. Kim, R. Jacob, N. Shastri, ERAAP customizes peptides for MHC class I molecules in the endoplasmic reticulum, Nature 419 (2002) 480–483.

[92] K. Falk, O. Rotzschke, S. Stevanovic, G. Jung, H.G. Rammensee, Allele-specific motifs revealed by sequencing of self-peptides eluted from MHC molecules, Nature 351 (1991) 290–296.

[93] H.G. Rammensee, Chemistry of peptides associated with MHC class I and class II molecules, Curr. Opin. Immunol. 7 (1995) 85–96.

[94] P.J. Bjorkman, M.A. Saper, B. Samraoui, W.S. Bennett, J.L. Strominger, D.C. Wiley, Structure of the human class I histocompatibility antigen, HLA-A2, Nature 329 (1987) 506–512.

[95] D.H. Fremont, M. Matsumura, E.A. Stura, P.A. Peterson, I.A. Wilson, Crystal structures of two viral peptides in complex with murine MHC class I H-2Kb, Science 257 (1992) 919–927.

[96] K. Natarajan, H. Li, R.A. Mariuzza, D.H. Margulies, MHC class I molecules, structure and function, Rev. Immunogenet. 1 (1999) 32–46.

[97] P. Cresswell, N. Bangia, T. Dick, G. Diedrich, The nature of the MHC class I peptide loading complex, Immunol. Rev. 172 (1999) 21–28.

[98] M.R. Farmery, N.J. Bulleid, Major histocompatibility class I folding, assembly, and degradation: a paradigm for two-stage quality control in the endoplasmic reticulum, Prog. Nucleic Acid Res. Mol. Biol. 67 (2001) 235–268.

[99] J.J. Neefjes, H.L. Ploegh, Allele and locus-specific differences in cell surface expression and the association of HLA class I heavy chain with beta 2-microglobulin: differential effects of inhibition of glycosylation on class I subunit association, Eur. J. Immunol. 18 (1988) 801–810.

[100] A.G. Grandea III, M.J. Androlewicz, R.S. Athwal, D.E. Geraghty, T. Spies, Dependence of peptide binding by MHC class I molecules on their interaction with TAP, Science 270 (1995) 105–108.

[101] C.A. Peh, S.R. Burrows, M. Barnden, R. Khanna, P. Cresswell, D.J. McCluskey, J. McCluskey, HLA-B27-restricted antigen presentation in the absence of tapasin reveals polymorphism in mechanisms of HLA class I peptide loading, Immunity 8 (1998) 531–542.

[102] E. Nossner, P. Parham, Species-specific differences in chaperone interaction of human and mouse major histocompatibility complex class I molecules, J. Exp. Med. 181 (1995) 327–337.

[103] E. Degen, M.F. Cohen-Doyle, D.B. Williams, Efficient dissociation of the p88 chaperone from major histocompatibility complex class I molecules requires both beta 2-microglobulin and peptide, J. Exp. Med. 175 (1992) 1653–1661.

[104] M.R. Jackson, M.F. Cohen-Doyle, P.A. Peterson, D.B. Williams, Regulation of MHC class I transport by the molecular chaperone, calnexin (p88, IP90), Science 263 (1994) 384–387.

[105] B. Ortmann, M.J. Androlewicz, P. Cresswell, MHC class I/beta 2-microglobulin complexes associate with TAP transporters before peptide binding, Nature 368 (1994) 864–867.

[106] K.M. Paulsson, P. Wang, P.O. Anderson, S. Chen, R.F. Pettersson, S. Li, Distinct differences in association of MHC class I with endoplasmic reticulum proteins in wild-type, and beta2-microglobulin- and TAP-deficient cell lines, Int. Immunol. 13 (2001) 1063–1073.

[107] J.L. Brodsky, R. Schekman, A Sec63p–BiP complex from yeast is required for protein translocation in a reconstituted proteoliposome, J. Cell Biol. 123 (1993) 1355–1363.

[108] F.E. Ware, A. Vassilakos, P.A. Peterson, M.R. Jackson, M.A. Lehrman, D.B. Williams, The molecular chaperone calnexin binds Glc1Man9GlcNAc2 oligosaccharide as an initial step in recognizing unfolded glycoproteins, J. Biol. Chem. 270 (1995) 4697–4704.

[109] E. Degen, D.B. Williams, Participation of a novel 88-kD protein in the biogenesis of murine class I histocompatibility molecules, J. Cell Biol. 112 (1991) 1099–1115.

[110] W.L. Maloy, Comparison of the primary structure of class I molecules, Immunol. Res. 6 (1987) 11–29.

[111] Q. Zhang, R.D. Salter, Distinct patterns of folding and interactions with calnexin and calreticulin in human class I MHC proteins with altered N-glycosylation, J. Immunol. 160 (1998) 831–837.

[112] E. Chevet, H.N. Wong, D. Gerber, C. Cochet, A. Fazel, P.H. Cameron, J.N. Gushue, D.Y. Thomas, J.J. Bergeron, Phosphorylation by CK2 and MAPK enhances calnexin association with ribosomes, EMBO J. 18 (1999) 3655–3666.

[113] E.T. Spiliotis, T. Pentcheva, M. Edidin, Probing for membrane domains in the endoplasmic reticulum: retention and degradation of unassembled MHC class I molecules, Mol. Biol. Cell 13 (2002) 1566–1581.

[114] S.A. Prasad, J.W. Yewdell, A. Porgador, B. Sadasivan, P. Cresswell, J.R. Bennink, Calnexin expression does not enhance the generation of MHC class I-peptide complexes, Eur. J. Immunol. 28 (1998) 907–913.

[115] B.K. Sadasivan, A. Cariappa, G.L. Waneck, P. Cresswell, Assembly, peptide loading, and transport of MHC class I molecules in a calnexin-negative cell line, Cold Spring Harbor Symp. Quant. Biol. 60 (1995) 267–275.

[116] D.B. Williams, Calnexin leads glycoproteins into the fold, Glycoconjugate J. 12 (1995) iii–iv.

[117] J.H. Weis, C. Murre, Differential expression of H-2Dd and H-2Ld histocompatibility antigens, J. Exp. Med. 161 (1985) 356–365.

[118] J.E. Scott, J.R. Dawson, MHC class I expression and transport in a calnexin-deficient cell line, J. Immunol. 155 (1995) 143–148.

[119] B. Gao, R. Adhikari, M. Howarth, K. Nakamura, M.C. Gold, A.B. Hill, R. Knee, M. Michalak, T. Elliott, Assembly and antigen-presenting function of MHC class I molecules in cells lacking the ER chaperone calreticulin, Immunity 16 (2002) 99–109.

[120] S.P. Srivastava, J.A. Fuchs, J.L. Holtzman, The reported cDNA sequence for phospholipase C alpha encodes protein disulfide isomerase, isozyme Q-2 and not phospholipase-C, Biochem. Biophys. Res. Commun. 193 (1993) 971–978.

[121] N. Hirano, F. Shibasaki, R. Sakai, T. Tanaka, J. Nishida, Y. Yazaki, T. Takenawa, H. Hirai, Molecular cloning of the human glucose-regulated protein ERp57/GRP58, a thiol-dependent reductase. Identification of its secretory form and inducible expression by the oncogenic transformation, Eur. J. Biochem. 234 (1995) 336–342.

[122] N.A. Morrice, S.J. Powis, A role for the thiol-dependent reductase ERp57 in the assembly of MHC class I molecules, Curr. Biol. 8 (1998) 713–716.

[123] J. Miyazaki, E. Appella, K. Ozato, Intracellular transport blockade caused by disruption of the disulfide bridge in the third external domain of major histocompatibility complex class I antigen, Proc. Natl. Acad. Sci. U. S. A. 83 (1986) 757–761.

[124] J.D. Smith, J.C. Solheim, B.M. Carreno, T.H. Hansen, Characterization of class I MHC folding intermediates and their disparate interactions with peptide and beta 2-microglobulin, Mol. Immunol. 32 (1995) 531–540.

[125] R.J. Warburton, M. Matsui, S.L. Rowland-Jones, M.C. Gammon, G.E. Katzenstein, T. Wei, M. Edidin, H.J. Zweerink, A.J. McMichael, J.A. Frelinger, Mutation of the alpha 2 domain disulfide bridge of the class I molecule HLA-A*0201. Effect on maturation and peptide presentation, Hum. Immunol. 39 (1994) 261–271.

[126] M.R. Farmery, S. Allen, A.J. Allen, N.J. Bulleid, The role of ERp57 in disulfide bond formation during the assembly of major histocompatibility complex class I in a synchronized semipermeabilized cell translation system, J. Biol. Chem. 275 (2000) 14933–14938.

[127] M. Tector, R.D. Salter, Calnexin influences folding of human class I histocompatibility proteins but not their assembly with beta 2-microglobulin, J. Biol. Chem. 270 (1995) 19638–19642.

[128] J.A. Lindquist, G.J. Hammerling, J. Trowsdale, ER60/ERp57 forms disulfide-bonded intermediates with MHC class I heavy chain, FASEB J. 15 (2001) 1448–1450.

[129] E. Rigney, M. Kojima, A. Glithero, T. Elliott, A soluble major histocompatibility complex class I peptide-binding platform undergoes a conformational change in response to peptide epitopes, J. Biol. Chem. 273 (1998) 14200–14204.

[130] T.P. Dick, N. Bangia, D.R. Peaper, P. Cresswell, Disulfide bond isomerization and the assembly of MHC class I–peptide complexes, Immunity 16 (2002) 87–98.

[131] B. Ortmann, J. Copeman, P.J. Lehner, B. Sadasivan, J.A. Herberg, A.G. Grandea, S.R. Riddell, R. Tampe, T. Spies, J. Trowsdale, P. Cresswell, A critical role for tapasin in the assembly and function of multimeric MHC class I–TAP complexes, Science 277 (1997) 1306–1309.

[132] Y. Li, L. Salter-Cid, A. Vitiello, T. Preckel, J.D. Lee, A. Angulo, Z. Cai, P.A. Peterson, Y. Yang, Regulation of transporter associated with antigen processing by phosphorylation, J. Biol. Chem. (2000).

[133] A.G. Grandea III, T.N. Golovina, S.E. Hamilton, V. Sriram, T. Spies, R.R. Brutkiewicz, J.T. Harty, L.C. Eisenlohr, L. Van Kaer, Impaired assembly yet normal trafficking of MHC class I molecules in Tapasin mutant mice, Immunity 13 (2000) 213–222.

[134] N. Garbi, P. Tan, A.D. Diehl, B.J. Chambers, H.G. Ljunggren, F. Momburg, G.J. Hammerling, Impaired immune responses and altered peptide repertoire in tapasin-deficient mice, Nat. Immunol. 1 (2000) 234–238.

[135] M.E. Paquet, D.B. Williams, Mutant MHC class I molecules define interactions between components of the peptide-loading complex, Int. Immunol. 14 (2002) 347–358.

[136] H.R. Turnquist, E.L. Schenk, M.M. McIlhaney, H.D. Hickman, W.H. Hildebrand, J.C. Solheim, Disparate binding of chaperone proteins by HLA-A subtypes, Immunogenetics 53 (2002) 830–834.

[137] J.C. Solheim, M.R. Harris, C.S. Kindle, T.H. Hansen, Prominence of beta 2-microglobulin, class I heavy chain conformation, and tapasin in the interactions of class I heavy chain with calreticulin and the transporter associated with antigen processing, J. Immunol. 158 (1997) 2236–2241.

[138] C. Soto, Protein misfolding and disease; protein refolding and therapy, FEBS Lett. 498 (2001) 204–207.

[139] A. Gruhler, K. Fruh, Control of MHC class I traffic from the endoplasmic reticulum by cellular chaperones and viral anti-chaperones, Traffic 1 (2000) 306–311.