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Comprehensive Invited Review

Molecular Mechanisms of MHC Class I-Antigen Processing: Redox Considerations

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Abstract

Major histocompatibility complex (MHC) class I molecules present antigenic peptides to the cell surface for screening by $CD8^+$ T cells. A number of ER-resident chaperones assist the assembly of peptides onto MHC class I molecules, a process that can be divided into several steps. Early folding of the MHC class I heavy chain is followed by its association with β_2-microglobulin (β_2m). The MHC class I heavy chain-β_2m heterodimer is

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incorporated into the peptide-loading complex, leading to peptide loading, release of the peptide-filled MHC class I molecules from the peptide-loading complex, and exit of the complete MHC class I complex from the ER. Because proper antigen presentation is vital for normal immune responses, the assembly of MHC class I molecules requires tight regulation. Emerging evidence indicates that thiol-based redox regulation plays critical roles in MHC class I–restricted antigen processing and presentation, establishing an unexpected link between redox biology and antigen processing. We review the influences of redox regulation on antigen processing and presentation. Because redox signaling pathways are a rich source of validated drug targets, newly discovered redox biology–mediated mechanisms of antigen processing may facilitate the development of more selective and therapeutic drugs or vaccines against immune diseases. *Antioxid. Redox Signal.* 11, 907–936.

I. Introduction

MHC class I molecules bind peptides derived from intracellular antigens in the ER and present them to the cell surface for recognition by $CD8^+$ cytolytic T lymphocytes (CTLs). Therefore, this antigen-recognition system is central to adaptive immune responses against tumors and virus-infected cells. The pathways of antigen processing convert cytosolic proteins into peptides and load these peptides onto MHC class I molecules for display on the cell surface to $CD8^+$ CTL. Over the past 30-year period, the discovery and characterization of the components of this antigen-processing machinery have provided a greater understanding of the cell biology of antigen processing and enabled the sequential event of antigen processing to be dissected into several individual steps. The five major events in this sequence are (a) generation of antigenic peptides by the proteolytic degradation of cytosolic proteins; (b) transport of the generated peptides into the ER; (c) initial folding and assembly of MHC class I molecules; (d) assembly of MHC class I–peptide complexes; and (e) surface expression of MHC class I–peptide complexes.

Antigen processing must be a highly regulated process that permits the induction of adequate $CD8^+$ CTL responses and, simultaneously, avoids the induction of unwanted immune responses that would result in autoimmunity (49, 106). Indeed, the folding, assembly, and loading of peptides into MHC class I molecules involve complex quality-control mechanisms (70). Recent studies show that thiol-based redox regulation plays an essential role in the multistep quality-control process of MHC class I assembly.

Redox regulation of antigen processing and presentation is an emerging field that has initiated the development of new concepts in immune regulation. In this review, we look at the recent progress made toward understanding the redox regulation in MHC class I–mediated antigen processing and presentation. We first outline the general immune responses that occur against foreign antigens, focusing on the classification of immune components and their specific functions. Next, we present an overview of the ER quality-control system, its redox environment, and the structure and function of oxidoreductases existing in the ER. We then discuss the assembly of MHC class I–peptide complexes, from the generation of antigenic peptides to the exit of MHC class I–peptide complexes from the ER. Ultimately, this review focuses on the roles of redox regulation in the initial folding and assembly of MHC class I molecules, assembly of the peptide-loading complex and optimal peptide loading, disassembly of the peptide-loading complex, and ER exit of MHC class I–peptide complexes. In addition, the importance of redox regulation in immune diseases is discussed. Understanding how MHC class I assembly is modulated by the ER redox-regulation system may enable us to design novel and more effective vaccines to combat challenging pathogens.

II. Elements of the Immune System and Their Functions

The immune system is a highly adaptable defense system that has evolved to protect organisms from invading pathogens. In vertebrates, the immune system can be subcategorized into either the innate or the adaptive immune response, based on the specificity and memory of the response, and these two arms of the immune system collaborate to protect the host efficiently (46, 73, 216). The innate immune response is characterized by broad reactivity and is the first line of defense against pathogens. For example, skin, as a physical barrier, interferes with the entry of pathogens (87, 182). Most components of innate immunity, such as leukocytes, natural killer cells, and proteins of the complement system, exist in the body before the host encounters an infectious agent and work together soon after the microbe infects the host. The innate immune system has broad reactivity and recognizes frequently encountered structures, called "pathogen-associated molecular patterns," that are characteristic of most microorganisms. The pathogen-associated molecular patterns are recognized by host pattern-recognition receptors (126). Of the well-characterized pattern-recognition receptors, one group is the toll-like receptors (177). Different toll-like receptors can detect lipopolysaccharides (LPSs) and peptidoglycans, components that are typically displayed on the surface of microorganisms; double-stranded RNA; unmethylated CpG DNA; and other derivatives of microorganisms (26, 27, 127, 232).

The adaptive immune response is a more extensive and specific immune response that recognizes and selectively eliminates specific microorganisms. Remarkably, the adaptive immunity distinguishes between self and nonself antigens and responds only to the foreign nonself antigens. Immunologic memory is also a prominent feature of the adaptive immune system (110, 148, 155). When a host encounters the same antigen a second time, the adaptive immune response against reinfection is faster and stronger than the primary immune response (277, 278). Thus, the adaptive immune system has a long-term memory of the pathogens it has previously encountered so that it can more efficiently eliminate those pathogens if they are ever encountered again.

The adaptive immune response consists of two branches, the humoral response (39, 77) and the cellular response (144). B lymphocytes participate in inducing humoral immune responses. When B cells interact with extracellular antigens through the B-cell receptor that is displayed on the cell surface, they proliferate and differentiate into antibody-secreting plasma cells (5, 80). Antibodies are the major effector molecules of the humoral immune response (305). The recognition of antigens by antibodies is highly specific, and the interaction often inhibits the activity of antigens or enhances the elimination of antigens by phagocytic cells (225).

The cell-mediated immune responses use T lymphocytes and antigen-presenting cells as the two major cellular components (33, 83, 299). Through maturation in the thymus, T cells express T-cell receptors (TCRs) on their surface. TCRs recognize antigens that are bound to major histocompatibility complex (MHC) molecules (57, 109). The MHC genome loci encode a tightly linked cluster of genes (29, 36, 137). The molecules encoded by these genes were found to be primarily responsible for mediating transplant-rejection reactions; thus, these genes were named "histocompatibility genes" (234, 257).

MHC molecules are divided into two major classes (Fig. 1). MHC class II molecules are expressed only by professional antigen-presenting cells, such as B cells, dendritic cells, and macrophages (154), and present exogenous antigens to $CD4^+$ T-helper cells (128). MHC class II molecules are composed of the α and β chains, which are type I transmembrane glycoproteins and associate through noncovalent interactions (34). MHC class I molecules are encoded by the K and D regions on mouse chromosome 17 and by the A, B, and C loci on human chromosome 6 (100, 229, 318). MHC class I molecules contain a 45-kDa heavy chain that associates through noncovalent interactions with the 12-kDa β_2m light chain. MHC class I molecules are expressed by almost all nucleated cells, loaded in the ER with peptides derived from both self and nonself intracellular proteins, and displayed on the cell surface for screening by $CD8^+$ CTLs (213, 263). Typically, MHC class I presents endogenously derived peptides, and MHC class II

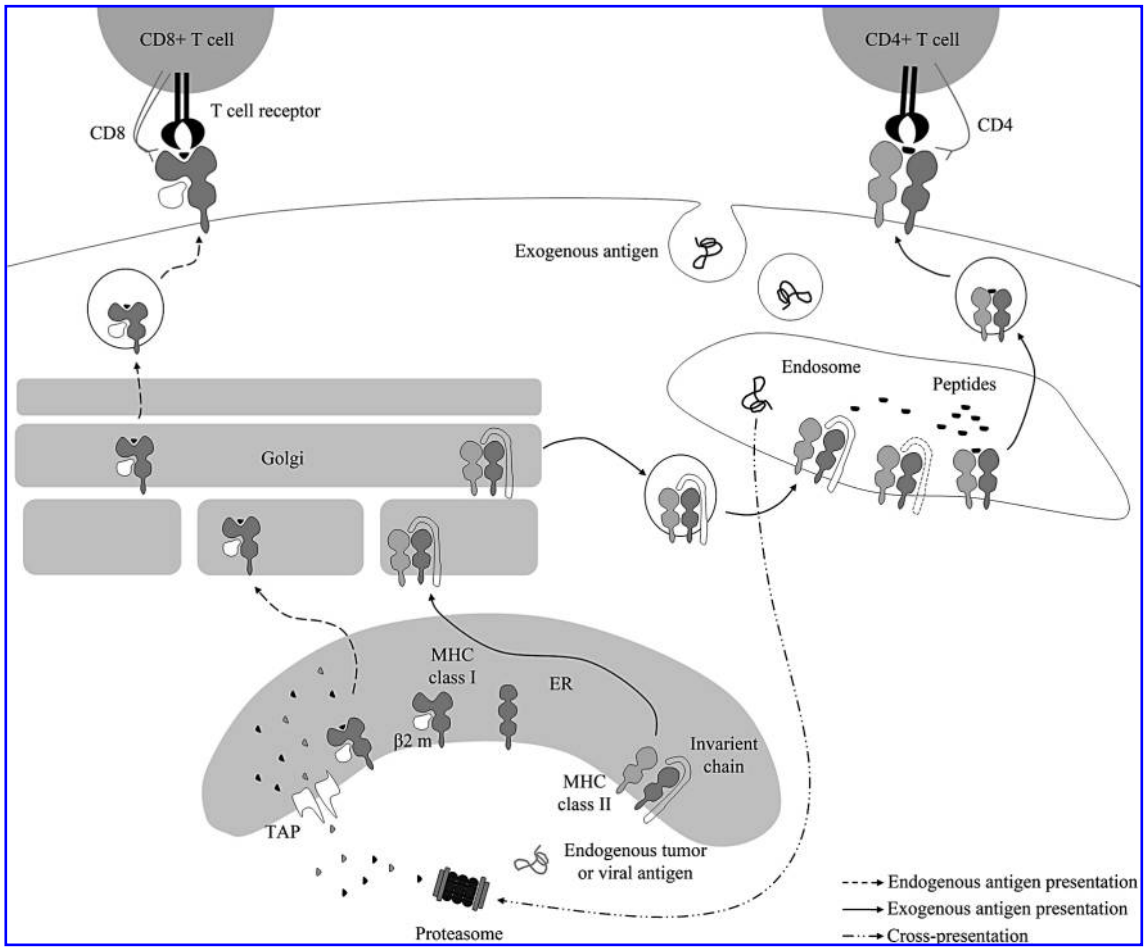


FIG. 1. Processing and presentation of endogenous and exogenous antigens. Endogenous antigens, such as viral or mutated proteins, are degraded into peptides by the proteasomes in the cytosol. Peptides are translocated into the ER, where they bind to MHC class I molecules. The peptide–MHC class I complexes go through the Golgi apparatus to the cell surface and then are recognized by $CD8^+$ T cells. Exogenous antigens are taken up by endocytosis or phagocytosis, enter endosomes, and are degraded into peptide fragments. MHC class II molecules are synthesized in the ER and transported to the endosomes, where they bind these peptides. The MHC class II–peptide complexes are delivered to the cell surface for recognition by $CD4^+$ T cells. Alternatively, exogenous antigens can be presented by MHC class I molecules for recognition by $CD8^+$ T cells, which is called "cross-presentation."

presents exogenously derived peptides. The presentation of exogenous antigens by MHC class I molecules also was reported and called "cross-presentation" (3, 4, 97, 233).

Normal cells constantly display their cytoplasmic protein contents on the cell surface by using MHC class I molecules. When cellular damage occurs or cells become infected with a virus, the repertoire of peptides loaded into MHC class I molecules is changed. For example, tumor-specific proteins are synthesized by cancerous cells. Similarly, viral proteins are expressed within virus-infected cells. In both cases, the repertoire of peptides would include representatives of the tumor-associated or viral proteins. The presentation of these foreign peptides induces a $CD8^+$ CTL response. Activated CTLs then specifically recognize and remove infected or cancer cells. The stimulation of CTLs by an antigen requires interaction between the T-cell receptor and the MHC class I molecule, as well as specific recognition of the loaded peptide (8, 169, 274). Hence, in addition to correct folding of the MHC class I molecule itself, the affinity between MHC class I molecules and peptide ligands is a key element that determines the nature and the outcome of the CTL response (43, 199). Only properly assembled MHC class I molecules are able to present peptides and induce appropriate CTL responses. If misfolded MHC class I molecules are not degraded and are expressed on the cell surface, they may initiate an abnormal CTL response against normal cells (239, 321) (Fig. 2). Thus, MHC class I assembly must be tightly regulated so that CTLs can accurately determine whether the cell is normal or unhealthy.

III. The Redox Environment of the Endoplasmic Reticulum and Protein Quality Control

A. Quality control of proteins in the ER

The ER is a protein-synthesizing and -packaging plant that is responsible for the biosynthesis, folding, assembly, and

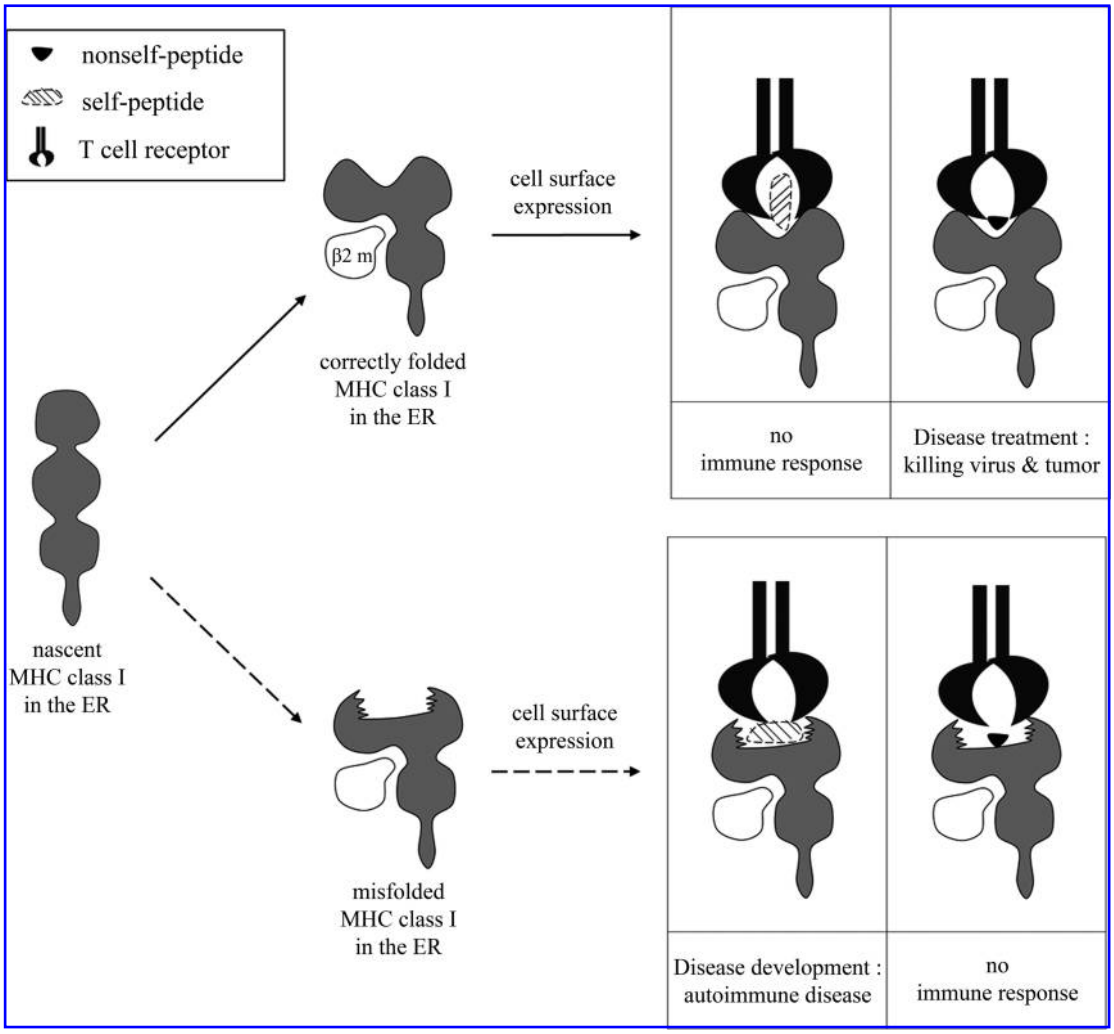


FIG. 2. MHC-mediated immunity: a double-edged sword. MHC-mediated immune responses can be either beneficial or detrimental, depending on the nature of the T-cell receptor (TCR)–MHC/peptide interaction. T cells normally recognize foreign peptides in the form of peptides displayed by MHC molecules and kill the virus-infected or cancerous cells. Transplantation rejection occurs because the MHC class I molecules of other tissues or organs are recognized as "nonself" despite their binding of "self" peptides. Misfolding of MHC class I molecules or inadequate peptide loading might initiate abnormal immune responses, such as autoimmune diseases. Therefore, the folding of MHC class I molecules and optimal peptide binding must be highly regulated.

modification of numerous soluble and membrane-bound proteins destined for secretion, for the cell surface, or for delivery to other intracellular organelles. The quality-control system makes sure that only correctly folded proteins are allowed to exit the ER for their final destinations (65, 260). Although the criteria by which folded and unfolded proteins are distinguished are still poorly characterized, the quality-control system seems to use conformational determinants, such as oligosaccharides, hydrophobic peptide elements exposed on the surface of proteins, and exposed free sulfhydryl groups as some of the indicators of a misfolded protein (108). Protein folding is assisted by a number of ER-resident chaperone molecules (185). During synthesis of proteins, the first chaperone protein that an unfolded polypeptide chain encounters is the binding immunoglobulin protein (BiP), a member of the Hsp70 family of chaperones (190). BiP facilitates the folding and assembly of newly synthesized proteins by recognizing unfolded polypeptides, keeping them in a competent state for subsequent folding and assembly (91). Because BiP has both an ATPase domain and a peptide-binding site, binding and release of polypeptides can be coupled to an ATP hydrolysis and ADP exchange cycle (91). Owing to the localization of BiP in the ER luminal side of the translocon, BiP both prevents the aggregation of cotranslationally imported but unfolded polypeptides and helps posttranslational import of proteins into the ER (105, 295). After being released by BiP, unfolded polypeptides of glycosylated proteins interact with the ER chaperone glucose-regulated protein 94 (GrP94) *en route* to the subsequent folding process (178). Calreticulin and calnexin are specialized ER lectin-binding chaperones, which bind transiently to newly synthesized glycoproteins (51, 65). Calreticulin and calnexin form a specific chaperone cycle, the "calreticulin–calnexin cycle," and cooperate to monitor the folding of glycoproteins *via* the successive calreticulin–calnexin cycle (66, 181). Even though calreticulin and calnexin show extensive sequence homology, only calreticulin interacts with the BiP-GrP94 complex (279) and recruits ER protein 57 (ERp57), which is a thiol oxidoreductase (156, 186). For the glycosylation of proteins, the core *N*-glycan is added to the polypeptide chain by an oligosaccharide transferase. Glucosidase 1 and 2 trim terminal glucose to generate a monoglucosylated glycan polypeptide (51, 58, 107, 302). The monoglucosylated glycan polypeptide then associates with calreticulin, calnexin, and the oxidoreductase ERp57 (42, 89, 187, 262). However, if the glycoprotein is misfolded, the terminal glucose is again attached by the action of UDP-glucose/glycoprotein glucosyltransferase (UGGT), which discriminates between folded and unfolded substrates (219). On completion of successful quality-control checks by calreticulin and calnexin, most glycopolypeptides exit the ER, whereas misfolded glycoproteins cannot escape from the calreticulin–calnexin cycle. The misfolded glycoprotein undergoes successive rounds of deglucosylation mediated by glucosidase 2 and reglucosylation through UDP-glucuronosyltransferase (UGT) (58, 186) until proper folding is achieved. If proper folding of the protein is never achieved, then the misfolded protein is retrotranslocated into the cytosol and eventually degraded by the proteasomes (66).

Disulfide bond formation plays a fundamental role in the folding and assembly of secretory and membrane proteins in the ER. In protein domains exposed to the lumen of the ER, almost all cysteines are disulfide bonded. Efficiently to form disulfide bonds in the ER, the lumen of the ER must sustain an oxidizing environment. The glutathione (GSH)/glutathione disulfide (GSSG) redox regulatory system primarily determines the cellular redox environment because glutathione has high intracellular abundance and relatively low redox potential (244). The redox environment of the ER is different from that of the cytosol. The ER maintains a relatively oxidizing environment (a GSH/GSSH ratio of 1:1 to 3:1), providing a redox potential favoring disulfide bond formation. In contrast, the redox state of cytosol is quite reducing, with a GSH/GSSH ratio of 30:1 to 100:1 (122, 163). If the oxidizing conditions in the ER are disturbed, oxidative protein folding that includes disulfide bond formation does not occur (31). Conversely, an oxidizing environment that is too strong will cause misfolding of proteins (172). Thus, maintaining homeostasis of the redox state in the ER is crucial in the quality control of proteins. Failure in the quality-control system causes unfolded proteins in the ER to accumulate, which induces the unfolded protein response (UPR) (319). Misfolded and incompletely assembled proteins are eventually destined for destruction by ER-associated degradation (ERAD) (66, 181).

B. Oxidoreductases and oxidative folding in the ER

The large number of oxidoreductases located in the ER indicates that the regulation of disulfide bond formation is crucial for protein folding and assembly. The most notable oxidoreductase in the ER is protein disulfide isomerase (PDI). PDI is a thiol-based oxidoreductase that catalyzes the oxidation, reduction, and isomerization of protein disulfide bonds (72, 200). PDI consists of four major domains with thioredoxin folds, denoted a, b, b′, and a′. The two catalytic a and a′ domains are separated by the two noncatalytic b and b′ domains (92). At its C-terminus, PDI has a short tail with calcium-binding properties (166). The a and a′ domains each contain an active site motif (CXXC), which is directly involved in thiol-disulfide exchange reactions during catalysis and is a well-known characteristic of the PDI family of proteins (75). The pK_a value of the active-site cysteine residues in each of the PDI family proteins determines the physiologic function of a particular PDI family member. The pK_a is determined by a conserved arginine residue located in the catalytic active site. This conserved arginine moves into and out of the active site, and this intradomain motion enables PDI to act as both an isomerase and oxidase (153).

The crystal structure of yeast PDI, which provides clues for understanding the functions and mechanisms of the proteins in the PDI family, reveals that the four thioredoxin domains are arranged in the "U" shape (281) (Fig. 3). The two catalytic sites face each other across the long sides of the U, and the inside surface of the U is rich in hydrophobic residues allowing misfolded proteins to be recognized by this surface. Because catalytic a and a′ domains and the noncatalytic b′ domain are perpendicular to each other, the substrates that accommodate to the b′ domain are positioned in spatial proximity to the two catalytic sites in the a and a′ domains and can undergo redox reactions (281). Biochemical studies suggest that all four domains of PDI are essential for full catalytic activity (52, 281). The relative contribution of each active site in PDI with regard to catalytic activity appears to be variable, depending on the substrates (201, 306).

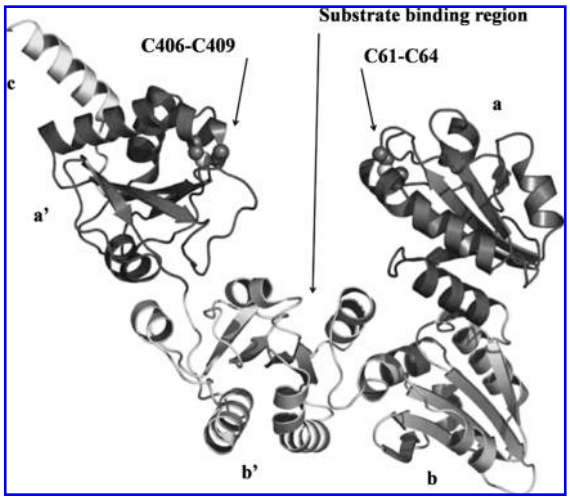


FIG. 3. X-ray crystal structure of yeast PDI. Yeast PDI exhibits a "U"-shaped structure. Catalytic active sites reside in the a and a′ domains, and the substrate-binding groove in the b′ domain is denoted by *arrows*. Active-site cysteines are shown in a space-filling representation. Reproduced with permission from reference 281.

The b′ domain of PDI provides the main site for binding substrates or peptides (44, 140). Particularly significant, PDI has a hydrophobic binding pocket in the b′ domain that recognizes small peptides of 10 to 15 amino acids (140, 226). Unlike PDI, ERp57, which is the closest structural and functional homologue of PDI, does not have a peptide-binding site, suggesting that ERp57 and PDI have distinct functions. Consistent with its ability to bind small peptides, PDI is the dominant acceptor for peptides translocated into the ER (151, 264). In addition to the unique ability of PDI to bind small peptides, another striking difference between PDI and ERp57 is that PDI can function as both an oxidase and reductase, whereas ERp57 has mainly reductase activity (271). Although the b′ domain in ERp57 is known to interact with *N*-glycoproteins *via* the P-domain of calreticulin during the calreticulin–calnexin cycle (82, 240), the substrate specificities of the b′ domain of other PDI family proteins have not yet been comprehensively characterized. Furthermore, the function of the b domain in the PDI family proteins is unknown, although the b domain is suggested to fulfill a purely structural requirement of PDI (52, 140).

To date, 17 PDI family proteins have been identified (Table 1). Only nine PDI family proteins possess thioredoxin-like catalytic a and a′ domains. Because an isomerase function appears to require not only catalytic a and a′ domains but also a noncatalytic b′ domain, the catalytic activities of ERp46, ERdj5, TMX, TMX3, and TMX4, which are devoid of a b′ domain, might be limited to oxidation and reduction but not isomerase activity (52). Interestingly, ERp18, which lacks the b′ domain, has recently been reported to have isomerase activity (129). Thus, the requirement of the b′ domain for isomerase activity will require further investigation. Whether some PDI family members work together on the same substrate or whether each protein acts on specific substrates remains unclear despite >30 years of investigation of the PDI proteins.

A major pathway for protein disulfide bond formation in the mammalian ER involves two ER proteins, PDI and endoplasmic reticulum oxidoreductin 1 (Ero1) (Fig. 4). Oxidized PDI can function as an electron acceptor, and thus as a disulfide donor, for the substrate proteins. When PDI catalyzes the oxidation of substrates, it becomes reduced. The redox state of PDI is regenerated by a series of direct thiol-disulfide exchange reactions that involve Ero1, its cofactor flavine adenine dinucleotide (FAD), and O_2 (255, 294). From the FAD cofactor, Ero1 derives oxidizing equivalents (16, 98, 288) that are transferred from Ero1 to PDI (81, 227, 289). Mammalian cells express two Ero1 proteins: hypoxia-inducible Ero1α (37, 90) and unfolded-protein-response-inducible Ero1β (210). PDI and ERp57 interact with these Ero1 proteins (90, 149, 210) and obtain oxidizing potential from Ero1 (90, 130, 149, 210).

IV. Antigen Processing and Presentation

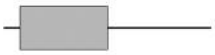
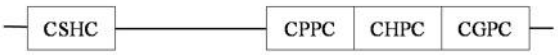
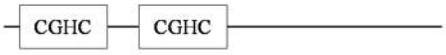
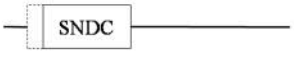
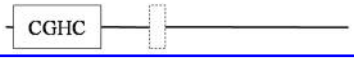
MHC class I molecules present antigenic peptides on the cell surface for recognition by $CD8^+$ CTL (Fig. 5). Like the folding of other glycoproteins, the folding and assembly of MHC class I molecules require interactions with a number of chaperone molecules in the ER, some of which are specific to MHC class I molecules (65). However, for MHC class I molecules, unlike for other glycoproteins, correct folding alone is not sufficient to trigger their exit from the ER. Instead, the MHC class I heavy chain can exit the ER only after it has been assembled with β_2m and loaded with a peptide (108, 311). The quality-control systems in the ER ensure that only properly assembled MHC class I–peptide complexes exit the ER and are transported to the cell surface (108). If MHC class I molecules cannot pass ER quality control, they are retrotranslocated to the cytosol and degraded (32). Therefore, the process of antigen processing, which consists of the folding, assembly, and peptide loading of MHC class I molecules, provides a particular example of the complex, multistep ER quality-control mechanisms that regulate antigen processing and subsequent MHC-mediated immune responses (66, 89).

A. The early assembly of MHC class I molecules

MHC class I molecules form trimeric complexes made up of the MHC class I heavy chain, β_2m, and the bound peptide (30). The MHC class I heavy chain is a highly variant and polymorphic type I transmembrane glycoprotein (215). Conversely, β_2m is relatively conserved and is not anchored to the membrane (25). The peptide is the antigenic element that is displayed on the cell surface in the context of the MHC class I complex.

On synthesis of the MHC class I heavy chain, the polypeptide is translocated to the ER membrane and binds calnexin (55, 56, 298) (Fig. 6). Calnexin is a membrane-associated chaperone with a lectin-binding site (107). The interaction between calnexin and the MHC class I molecule might stabilize the class I heavy chain and help it to associate with the β_2m component. ERp57 also associates with class I heavy chains that are bound to calnexin (320). Once the MHC class I heavy chain is assembled into a heterodimer with β_2m, the complex dissociates from calnexin (62). The class I heavy chain–β_2m heterodimer then binds to calreticulin (69) and is incorporated into the peptide-loading complex.

Table 1. Protein Disulfide Isomerase Family Members Presents in the ER

Protein (A.N.O)	*Domain architecture*	*Activity*	*Function*
PDI (P07237)		Oxidase Reductase Isomerase Chaperone	PLC component(218,243) Protein retrotranslocation(79) Anti-inflammatory(322) Protein retention in the ER(208) Component of collagen biosynthesis(313)
ERp57 (P30101)		Oxidase Reductase	PLC component(138,243,268) Molecular marker of immunogenicity(203)
PDIp (Q13087)		Oxidase Reductase Chaperone	Peptide binding(139) Neurodegeneration(47)
ERp72 (P13667)		Oxidase Reductase Isomerase Chaperone	ER retention(79)
PDILT (Q8N807)		unknown	Unknown(297)*
ERp27 (Q96DN0)		unknown	Unknown(10)*
PDIr (Q14554)		Reductase Isomerase Chaperone	Unknown(112)*
ERp28 (P30040)		unknown	Unknown(74)*
ERdj5 (Q8IXB1)		Reductase	ERAD(63,293)
P5 (Q15084)		Oxidase Isomerase Chaperone	Tumor immune evasion(133)
ERp18 (O95881)		Oxidase Isomerase	Apoptosis against ER stress(129)
ERp44 (Q9BS26)		unknown	Protein retention in the ER(15,208) Regulation of IP3R1 activity(117)
ERp46 (Q8NBS9)		unknown	Unknown(143)*
TMX (Q9H3N1)		Reductase	Reduction of ER stress (175)
TMX2 (Q9Y320)		Unknown	Unknown(179)*
TMX3 (Q96JJ7)		Oxidase	Unknown(111)*
TMX4 (Q9HIE5)		unknown	Unknown

Open rectangles represent thioredoxin-like a and a′ domains with catalytic active sites. The catalytically inactive b domain is represented by a light-gray rectangle; the other b′ domain is represented by a dark-gray rectangle. The black rectangle between the b′ and a′ domains represents the linker domains, and rectangles demarcated with a dotted line indicate transmembrane domains. A solid-line oval attached to the carboxy-terminal tail of PDI represents an acidic extension, whereas a dotted-line oval attached to the carboxy-terminal tail of ERp57 represents a basic extension. Stars mean references for protein characterization.

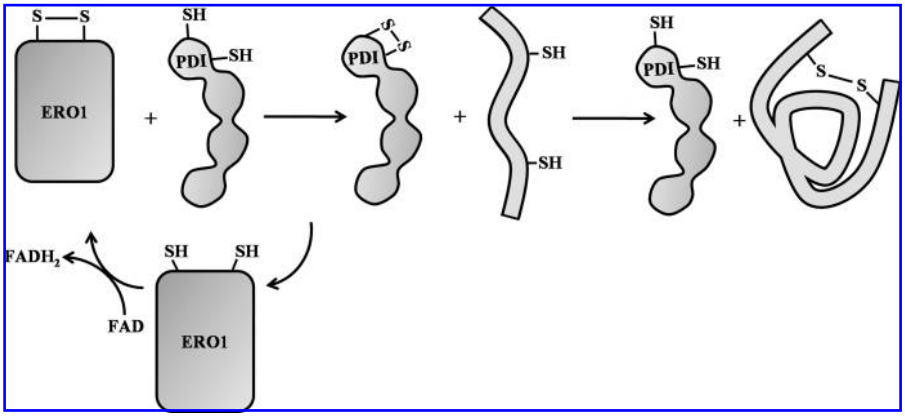


FIG. 4. Dithiol-disulfide exchange mechanism. PDI-catalyzed disulfide bond formation involves several steps of protein–protein interactions. PDI is oxidized through dithiol groups and disulfide bond exchange between PDI and Ero1. Next, oxidized PDI generates proper oxidized target proteins through thiol group and disulfide bond exchange between PDI and target proteins. Formation of disulfide bonds flows from Ero1 to PDI and from PDI to the secretory target protein, whereas the flow of the electrons is opposite. The oxidizing potential of Ero1 is transferred from FAD molecules.

B. Generation of antigenic peptides and peptide translocation into the ER

The usual turnover of cellular proteins generates peptides that are used for immune surveillance by T cells. Peptides are produced mainly through the ubiquitin–proteasome pathway. Ubiquitin, a well-known component of the ubiquitin–proteasome pathway that is involved in regulating protein turnover (116), is a small protein that is composed of 76 amino acids and is highly conserved. For attachment of ubiquitin to substrates, called ubiquitination or ubiquitinylation, three major enzymes participate. E1 ubiquitin-activating enzyme

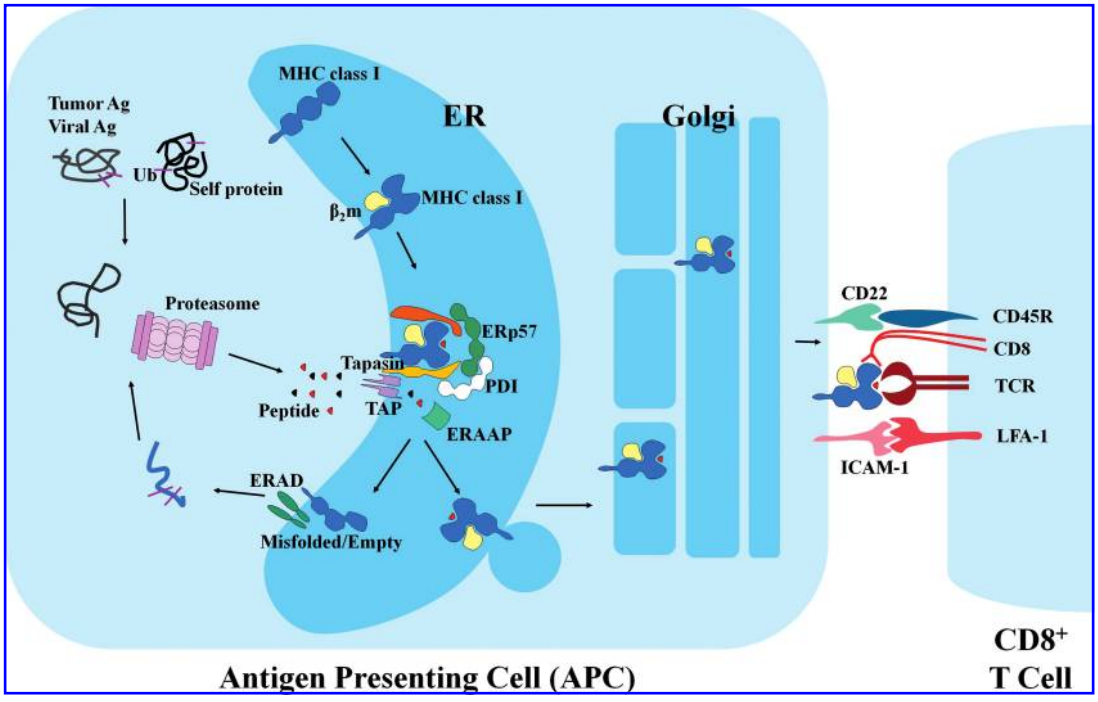


FIG. 5. Pathway of MHC class I antigen processing and presentation. The newly synthesized MHC class I heavy chain is initially folded with the help of several chaperones (calnexin, BiP, ERp57) and then associates with β_2m. This MHC class I heterodimer enters the peptide-loading complex, and various components (ERp57, tapasin, TAP, calreticulin, and PDI) regulate peptide loading onto MHC class I molecules. Endogenous proteins are degraded by proteasomes to generate peptides that are transported into the ER through TAP. Transported peptides are trimmed further by ERAAP and loaded onto MHC class I molecules. Stable MHC class I heterotrimers that pass the ER quality-control systems are transported from the ER to the cell surface by the Golgi apparatus. If MHC class I molecules do not pass the ER quality-control system, they are retrotranslocated to the cytosol and degraded by the proteasomes. MHC class I molecules that are presented on the cell surface are scanned by T cells. The T-cell receptor recognizes the MHC class I molecule, and CD8 functions as a co-receptor. Other accessory molecules (CD22, CD45R, ICAM-1, and LFA-1) facilitate this process. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars).

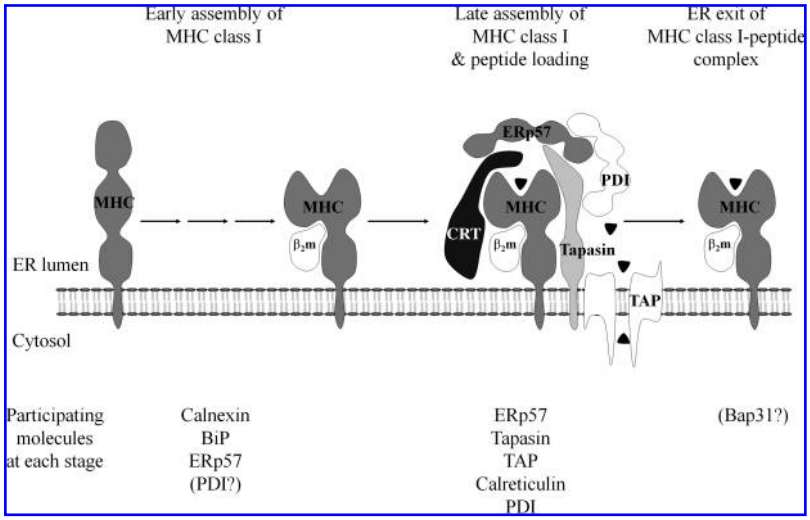


FIG. 6. Early and late assembly of MHC class I molecules. During the early assembly stage, nascent MHC class I heavy chain interacts with several chaperone molecules (calnexin, BiP, and ERp57). These molecules help the correct folding of the MHC class I molecule. PDI also is thought to be involved in this stage. After being released from calnexin, the MHC class I heavy chain associates with the β_2m subunit. The MHC class I–β_2m heterodimer is then integrated into the peptide-loading complex. Peptide loading onto the MHC class I molecules is facilitated by various components of the peptide-loading complex (ERp57, tapasin, TAP, calreticulin, and PDI). In this late assembly stage, the MHC class I molecule is systemically regulated to load optimal peptides by these components.

catalyzes the ATP-dependent formation of a thioester linkage between the C-terminal glycine of ubiquitin and the active-site cysteine of E1 (102, 103, 310). Formation of ubiquitin-E1 conjugate is followed by the transfer of ubiquitin from E1 to the active-site cysteine of the E2 ubiquitin-conjugating enzyme (245). Finally, ubiquitin is conjugated to substrate by E3 ubiquitin ligase, which can interact with both E2 and substrate (115).

The ubiquitin-conjugated substrates are degraded by the proteasome. The proteasome, a multisubunit complex in the cytosol that has a broad spectrum of proteolytic activities, is found in virtually all organisms (114, 317). The 26S proteasome consists of two subunits, the 19S cap complex and the 20S core particle (23). The 19S cap complex is the regulatory particle and is responsible for recognizing the poly-Ub chain on substrates. It is generally believed that substrates must be denatured or unfolded before entering into the proteasome (95, 164). The 20S proteasome is composed of two outer rings of α subunits and two inner rings of β subunits (96). The synthesis of three of the proteasome subunits, LMP2, LMP7, and MECL-1, is induced on treatment of cells with interferon-γ, which is typically produced during a virus infection (237, 276). These three subunits replace constitutive subunits β_1, β_5, and β_2 of the proteasome; this replacement results in the formation of the immunoproteasome (85). Interferon-γ also increases the expression of the PA28α/β complex that interacts with the 20S proteasome and thereby facilitates the proteolytic activity of the 20S proteasome (193). Thymic epithelial cells express "thymoproteasome," besides constitutive and immunoproteasomes. The catalytic subunit β_{5t} replaces the constitutive β_5 subunit in thymoproteasome. Thymoproteasome has significantly reduced chymotrypsin activity (191) and generates a unique set of self-peptides that are seen only in thymic epithelial cells and are pivotal for positive selection of $CD8^+$ T cells (192). The immunoproteasome specifically cleaves polypeptides after hydrophobic residues (64, 86). This activity is crucial because most MHC class I molecules preferentially bind peptides that have hydrophobic carboxy termini (220). Although the carboxy terminus of antigenic peptides is generated entirely through proteasomal cleavage, the amino terminus of peptides is not suitably cleaved by the proteasome, and additional cleavage steps are needed to trim the amino terminus of peptides before they can be loaded into the MHC class I complex (141). Several cytosolic proteases, such as leucine aminopeptidase (LAP) (24), bleomycin hydrolase (BH) (284), puromycin-sensitive aminopeptidase (PSA) (269), and tripeptidyl peptidase II (TPP II) (300, 315), have been proposed to be responsible for the trimming of the amino terminus of antigenic peptides. However, recent studies show that antigen processing and the MHC class I level is normal in PSA-deficient cell and that PSA-deficient mice display a normal T-cell response (283). LAP-deficient mice also exhibit normal phenotypes regarding T-cell responses (282). Therefore, the functions of these cytosolic proteases in antigen processing require further clarification. Nevertheless, these peptides either are transported into the ER through TAP to be loaded into MHC class I complexes or they are eventually broken down into individual amino acids in the cytosol.

TAP is a heterodimeric complex, which is composed of the TAP1 and TAP2 proteins, and is part of the ATP-binding cassette (ABC) superfamily (1). TAP consists of two transmembrane domains and two cytosolic nucleotide-binding domains (146). Peptides are translocated from the cytosol to the ER lumen through TAP by a two-step process: (a) ATP-independent peptide binding to TAP and (b) ATP-dependent translocation of peptides (183, 198). Peptide binding to TAP causes it to undergo a conformational change that induces ATP hydrolysis, forcing the opening of a pore and translocation of the peptide into the ER lumen (248, 296). The transmembrane domains play a role in dimerization of TAP1 and 2 and are necessary to target the complex to the ER (145). Furthermore, transmembrane domains are crucial for the association of TAP with tapasin and the assembly of the peptide-loading complex (146). The first three residues at the amino terminus and the last residue at the carboxy terminus of peptides are critical for their binding to TAP (250). Peptides with hydrophobic C-terminal residues interact more efficiently with TAP, consistent with the specificity of the peptide-binding domain of MHC class I molecules (189). Although the TAP complex has the structural flexibility to recognize a variety of substrates, TAP prefers peptides with eight to 16 amino acids, which is the peptide length typically generated by the immunoproteasome (292).

Because the peptide-binding pocket of MHC class I prefers peptides with a length of eight to 10 amino acids, most TAP-translocated peptides must be trimmed before being loaded onto MHC class I molecules. Recent studies indicate that ER aminopeptidase associated with antigen processing (ERAAP) is the key enzyme for polishing of the peptides (41, 254). ERAAP tends to trim peptides to a length of eight to 10 amino acids and cleaves peptides longer than 10 amino acids at a high rate, but it is rarely active on peptides shorter than eight amino acids (316). The functional specificity of ERAAP generates peptides that are well suited to be loaded into MHC class I complexes.

C. The assembly of the peptide-loading complex and peptide loading

MHC class I–β_2m heterodimers are recruited into the peptide-loading complex, a multiprotein complex that includes calreticulin, ERp57, TAP, and tapasin (18, 296) (Fig. 6). Unlike ERp57 and calreticulin, which are part of the general protein quality-control process, TAP and tapasin are dedicated to the quality control of MHC class I assembly. Peptides are loaded onto the MHC class I molecules in the peptide-loading complex, and individual components cooperate to stabilize the MHC class I complex and to load optimally structured peptides. Tapasin is a type I transmembrane protein and directly interacts with TAP to form a physical bridge between TAP and the MHC class I molecules, indicating that tapasin maintains the structural integrity of the peptide-loading complex (206, 242). Moreover, tapasin appears to be involved in facilitating optimal peptide loading (312). Interestingly, the influence of tapasin on the cell-surface expression of MHC class I molecules is allele specific (223). Allele specificity of tapasin dependence seems to relate closely to the nature of the amino acid residue at position 114 or 116 of MHC class I heavy chains (217, 312). HLA-B*4402 is different from HLA-B*4405 by a single amino acid. HLA-B*4402 has asparagine at position 116 and depends on tapasin for peptide loading (312). Conversely, HLA-B*4405 has tyrosine at the same position and is not affected by the absence of tapasin for its cell-surface expression (259, 312). Similarly, a single amino acid at the position 114 affects allele specificity of tapasin. Substitution of histidine by glutamic acid at the position 114 switches HLA-B*4402 into a tapasin-independent allele. Reverse substitution, histidine to glutamic acid at the same position, makes tapasin-independent HLA-B*2705 switch to tapasin dependence (217). However, the mechanisms by which tapasin facilitates peptide loading remain incompletely characterized.

ERp57 associates with either calnexin or calreticulin, suggesting that ERp57 might participate in disulfide bond formation and accurate folding of monoglucosylated polypeptides in the ER (136, 188, 205). Because of its association with calnexin and calreticulin, ERp57 is involved in both the early and late steps for the assembly of MHC class I complexes (69, 120). In the peptide-loading complex, ERp57 forms a disulfide-linked conjugate with tapasin; the exact function of ERp57 in antigen processing is unclear, but ERp57 activity appears to have species-to-species variations (56, 61, 202, 222).

One recent study shows that ERp57 functions in MHC class I antigen presentation, but its catalytic activity does not affect MHC class I maturation and folding (221). Further, our group recently identified PDI as a component of the peptide-loading complex (218). This work demonstrates the existence of transient MHC class I–PDI disulfide intermediates in the peptide-loading complex. Within these intermediates, PDI catalyzes oxidation of the α2 disulfide bond within the peptide-binding groove of MHC class I molecules, and this function was essential for optimal peptide loading by MHC class I molecules (218).

Calreticulin, a soluble homologue of calnexin (65) and a protein that is important for calcium homeostasis and the folding of glycoproteins (180), is also found in the peptide-loading complex (242). The function of calreticulin in the peptide-loading complex is largely unknown. Calreticulin binds to oxidized MHC class I molecules only after the MHC class I heavy chain–β_2m dimer has assembled but before a peptide has been loaded (69, 242). In a calreticulin-deficient cell line, MHC class I molecules assemble with β_2m normally, but the subsequent loading of a peptide onto the heterodimer is defective (88). The most plausible mechanism by which calreticulin facilitates loading of optimally structured peptides onto MHC class I complexes is to stabilize the interactions among the various components of the peptide-loading complex, an explanation that is supported by several studies (158, 160, 223, 242). Calreticulin-mediated stabilization would minimize the release of prematured MHC class I molecules from the peptide-loading complex, giving the MHC class I molecules a better chance to have an optimally structured peptide loaded into the antigen groove.

V. Redox Regulation of Early Folding and Assembly of MHC Class I Molecules

A. Oxidative folding of MHC class I molecules

Disulfide bond formation is essential for maintaining the structure and the function of proteins. The structure of the MHC class I molecules consists of a membrane-distal region containing the α_1 and α_2 domains of the heavy chain and a membrane-proximal region containing the α_3 domain of the heavy chain and the β_2m subunit (30). The peptide-binding groove is made up of the α_1 and α2 domains, wherein the α_2 domain contains two cysteine residues, cys^{101} and cys^{164}. The α_3 domain also has two cysteine residues, cys^{203} and cys^{259}, and this domain interacts extensively with β_2m. Cys^{101} and cys^{164} in the α_2 domain and cys^{203} and cys^{259} in the α_3 domain form intradomain disulfide bonds (30) (Fig. 7). These cysteine residues are highly conserved among MHC class I alleles, whereas the cysteine residues in the cytoplasmic tail are poorly conserved and do not form intradomain disulfide bonds (Fig. 8). In the α_3 domain, the disulfide bond is rapidly formed after synthesis of MHC class I molecules in the ER and is necessary for this domain to adopt the immunoglobulin fold (236, 301). Once formed, this disulfide bond is very stable because it is buried in the hydrophobic core of the MHC class I heavy chain (104, 123) and shielded by the β_2m subunit (30). In contrast, the disulfide bond within the α_2 domain is located in the peptide-binding groove and is unstable before peptide loading. Hence, empty MHC class I molecules are presumably vulnerable to attack by oxidoreductases such as PDI and ERp57. Consistent with this assumption, an *in vitro* study showed that in the α_2 domain of unassembled MHC class I molecules, the disulfide bond is efficiently reduced by ERp57, whereas fully assembled MHC class I molecules are resistant

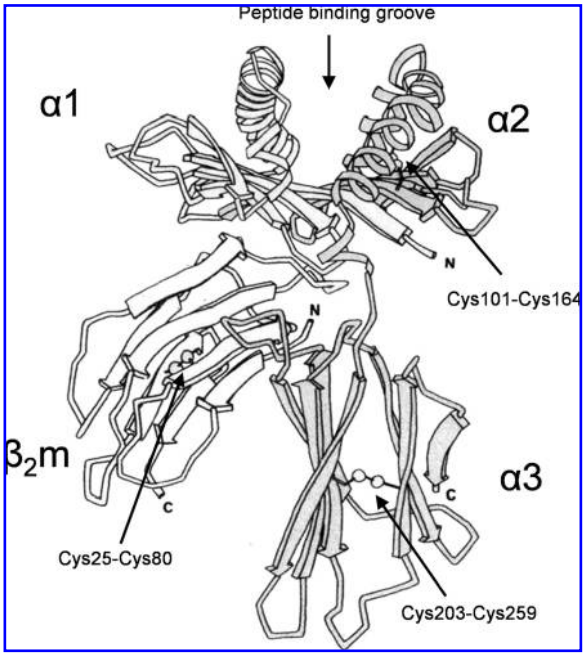


FIG. 7. Disulfide bonds in MHC class I molecules. The MHC class I molecule consists of the heavy chain that is subdivided into the α_1, α_2, and α_3 domains and the light chain, β_2m. The α_2 domain, α_3 domain, and β_2m have intramolecular disulfide bonds. The peptide-binding groove is located between the α_1 and α_2 domains. Disulfide bonds are indicated by two connected spheres. Reproduced with permission from reference 30.

to the reductase activity of ERp57 (16). Further, in HeLa cells, blocking the delivery of peptides into the ER increased the ability to reduce the disulfide in the α_2 domain of MHC class I molecules (218).

As an adjunct to the biochemical studies, mutational analysis of MHC class I molecules suggests that the thiol-mediated folding of MHC class I molecules is crucial for the efficient antigen presentation. If the cysteine residues that participate in the disulfide bond are substituted with alanine or serine, the resulting C101S or C164A MHC class I mutants fail to form the disulfide bond and are not expressed on the cell surface (258, 261). Further, HLA-A2.1 mutants that lack a disulfide bond in the α_2 domain assemble inefficiently with β_2m (303). Moreover, α_2 or α_3 domain cysteine mutants of HLA-A2.1 are quickly degraded (unpublished observation).

B. Redox regulation of MHC class I molecules in the early folding stage

The MHC class I heavy chain is folded before its integration into the peptide-loading complex. This initial folding, the "early folding stage," involves interactions between MHC class I heavy chain and calnexin, the oxidoreductase ERp57, and β_2m (18). Calnexin binds to newly synthesized, free MHC class I heavy chain *via* an interaction with lectin, facilitating the folding of the MHC class I heavy chain and preventing its aggregation (270, 280). The interaction of ERp57 with the native MHC class I heavy chain in the early folding stage suggests a role for ERp57 in disulfide bond formation of the MHC class I heavy chain (69). This activity of ERp57 was demonstrated by the depletion of ERp57 by RNA interference in mouse L cells, which resulted in delayed MHC class I heavy-chain disulfide bond formation and slowed folding of the heavy-chain α_3 domain (320). Interestingly, the association between MHC class I heavy chain and β_2m is not altered in ERp57-depleted cells, suggesting that the interaction between heavy chain and β_2m does not depend on the redox state of cysteine residues in the α_3 domain (320). Although these data demonstrate a role for ERp57 in disulfide bond formation in the α_3 domain, the effect of ERp57 knockdown on the disulfide bond in the α_2 domain was not analyzed. Of note, analysis of ERp57-deficient mice showed that the lack of ERp57 does not influence the redox state of MHC class I molecules (89). The apparent discrepancies between these two studies could be a

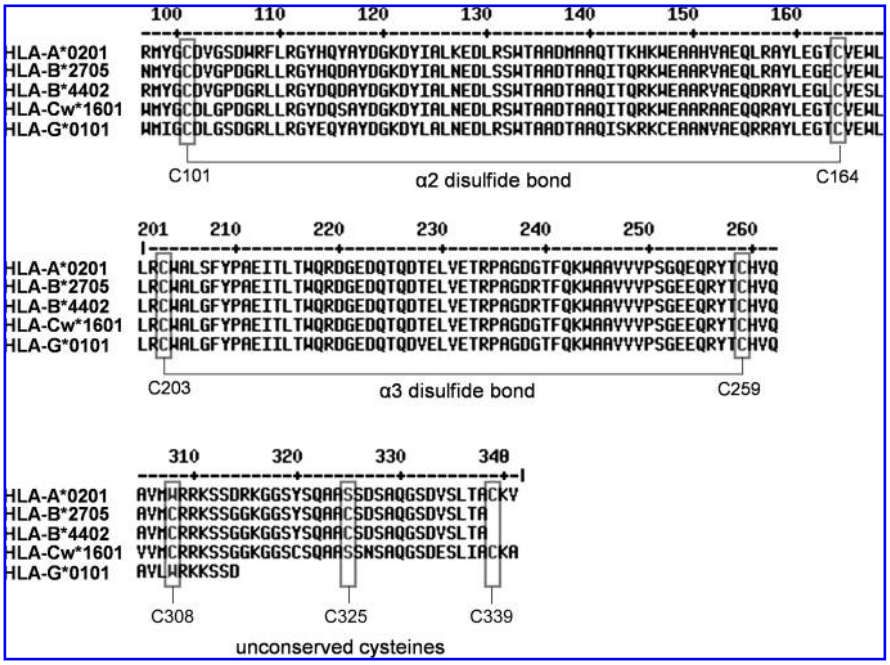


FIG. 8. Conserved cysteines in MHC class I molecules. Multiple sequence alignment of the MHC class I alleles. Cysteine residues are boxed. Residues cys^{101} and cys^{164}, which form a disulfide bond in the α_2 domain, and residues cys^{203} and cys259, which form a disulfide bond in the α_3 domain, are conserved among most of the MHC class I alleles. Cys^{308}, cys^{325}, and cys^{339} at the carboxy termini are not conserved.

result of different experimental conditions. For example, in the former study, a small pool of MHC class I heavy chain in the early folding stage was analyzed with pulse-chase studies (320), whereas in the latter study, the whole pool of MHC class I heavy chain was analyzed by immunoblotting (89).

Additional molecules are possibly involved in the early oxidative folding of MHC class I molecules. Our recent work indicates that PDI may be one candidate important for the early folding stage of the MHC class I heavy chain. PDI forms a disulfide intermediate with the α_2 domain of the MHC class I heavy chain both within the peptide-loading complex and independent of the peptide-loading complex (218). The mixed disulfide intermediate between the MHC class I heavy chain and PDI found outside the peptide-loading complex may represent a transient form of the MHC class I molecules undergoing early oxidative folding.

VI. Redox Regulation of the Peptide-Loading Complex Assembly and Peptide Loading

A. Redox network in the peptide-loading complex

Several proteins, including TAP, tapasin, ERp57, calreticulin, and the class I heavy chain–β_2m complex, compose the peptide-loading complex (78). Recently, PDI was identified as another component of the peptide-loading complex (218, 243). These components have specific functions and structural properties critical for antigen processing. Of particular significance to this review, several components of the peptide-loading complex, such as the MHC class I heavy chain, ERp57, tapasin, and PDI, are linked through the disulfide network (16, 61, 218, 290). Each of these proteins contains cysteine residues that can participate in the formation of the intra- or intermolecular disulfide bonds. Formation and dissociation of some of these disulfide bonds are reciprocally regulated among the components by various mechanisms. This section details the redox network in the peptide-loading complex (Fig. 9).

The MHC class I heavy chain forms mixed disulfide complexes with multiple components of the peptide-loading complex. Currently, three disulfide-interaction partners for MHC class I molecules are known. First, the MHC class I heavy chain forms a disulfide intermediate with ERp57 (162). Powis and co-workers (16) observed that ERp57 forms a mixed disulfide intermediate with free class I heavy chain and preferentially reduced partially folded MHC class I molecules. In contrast, correctly folded and peptide-loaded MHC class I molecules were resistant to ERp57 reduction (16). However, no direct evidence suggests that an MHC class I

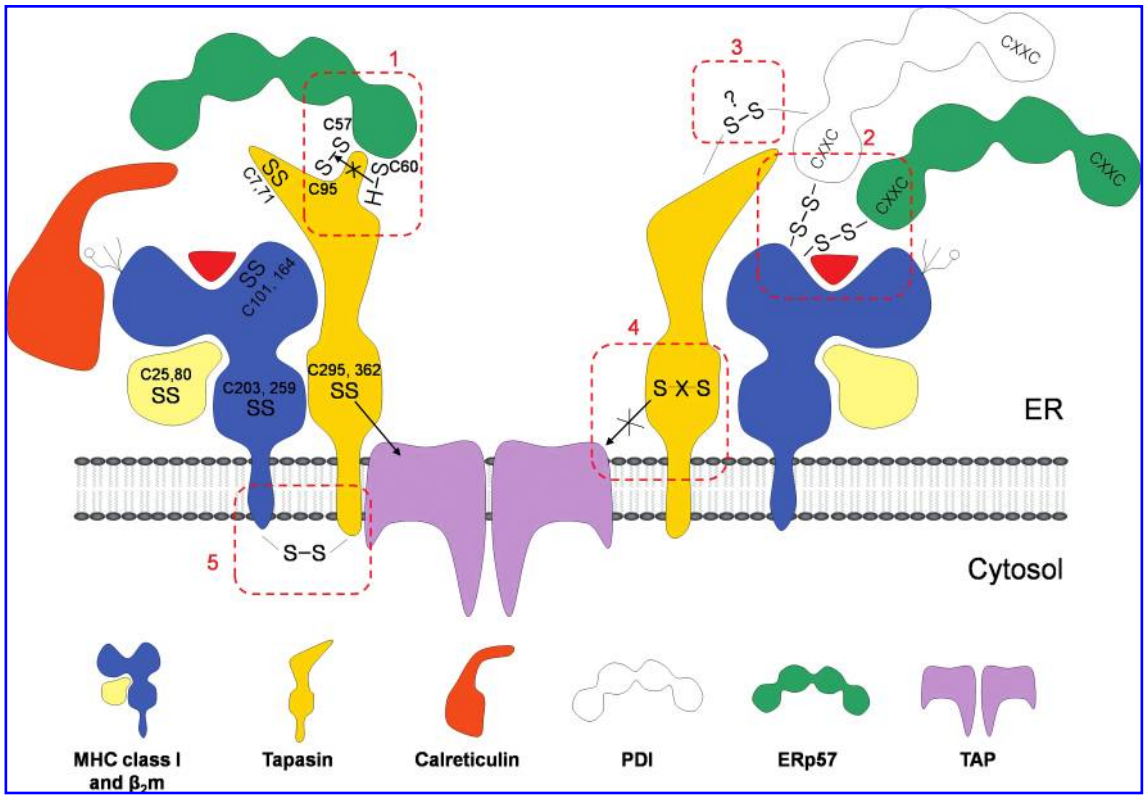


FIG. 9. Schematic representation of the redox network in the peptide-loading complex. (**1**) Cys^{57} of ERp57 and cys^{95} of tapasin form an intermolecular disulfide bond. Noncovalent interactions between ERp57 and tapasin inactivate the escape pathway mediated by cys^{60} of ERp57. (**2**) The α_2 domains of the MHC class I molecule form disulfide intermediates with PDI and ERp57. (**3**) PDI and tapasin form a transient intermolecular disulfide bond. Noncovalent interaction between PDI and tapasin also exists. (**4**) Cys^{295} and cys^{362} of tapasin form an intramolecular disulfide bond. Unfolded tapasin cannot interact with the TAP complex. (**5**) Cys^{420} of tapasin participates in formation of an intermolecular disulfide bond with cys^{308} in MHC class I molecules. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars).

heavy chain–ERp57 conjugate exists within the peptide-loading complex. This absence implies that the MHC class I heavy chain–ERp57 conjugate may represent an intermediate that is *en route* to the degradation pathways. The second mixed disulfide partner is PDI. MHC class I heavy chain forms disulfide intermediates with PDI both inside and outside the peptide-loading complex (218). The function of PDI in the peptide-loading complex has been well characterized; however, the function of PDI outside the peptide-loading complex is unknown. Mutational analysis showed that PDI forms intermediates with the two cysteine residues in the α_2 domain of the MHC class I heavy chain (218). The third partner known is tapasin. A recent report used a semi-permeabilized cell-translation system to show that cys^{308} in the carboxy terminus of MHC class I heavy chain is disulfide linked with tapasin (40). Intriguingly, this interaction cannot be detected in a TAP-negative cell line, and the physiological meaning of this interaction must be studied further.

ERp57, which is also an integral component of the peptide-loading complex, forms a very stable disulfide bond with tapasin within the peptide-loading complex (61, 222). This conjugate can be detected either by treating cells with the sulfhydryl-reactive reagent *N*-ethylmaleimide (NEM) or methyl methanethiosulfonate (MMTS) or by expressing the trapping mutant ERp57-C60A in the absence of NEM or MMTS. This interaction is mediated between cys^{57} of ERp57 and cys^{95} of tapasin (61). Further, several lines of evidence suggest that the structure of tapasin affects formation of the proper ERp57–tapasin conjugate. First, oxidation of tapasin is coupled with the formation of the ERp57–tapasin complex within the peptide-loading complex. Additionally, mutagenesis disrupting the intramolecular disulfide bond between cys^{7} and cys^{71} in tapasin reduces the formation of the ERp57–tapasin conjugate (61). Proper formation of this conjugate is independent of β_2m expression and the presence of monoglucosylated *N*-linked glycans (222). The high degree of stability of the ERp57–tapasin conjugate is unique because disulfide intermediates between substrate proteins and members of the thioredoxin family proteins are typically transient, and the escape pathway prevents accumulation of conjugates (255). However, once the ERp57–tapasin conjugate is formed in the peptide-loading complex, it is sequestered through a noncovalent interaction between tapasin and ERp57 that inhibits the reducing activity of cys^{60} in ERp57 (222). This inhibition leads to the inactivation of the escape pathway, preventing the reduction of this bond (221).

Tapasin has five conserved cysteines. As described earlier, cys^{7} and cys^{71} form an intramolecular disulfide bond, and cys^{95} is required for conjugation to ERp57 (61). The other two cysteines, cys^{295} and cys^{362}, are located in immunoglobulin (Ig)-like domain. Mutational analysis suggests that the disulfide bond in this Ig-like domain is critical for the structural stability of tapasin. Mutation of these cysteines abrogates the interaction between tapasin and MHC class I molecules (290). These mutations reduce the stability of TAP and the interaction between tapasin and TAP (290). Moreover, we found that tapasin is disulfide conjugated to PDI (unpublished observation). Although a functional role for the PDI–tapasin disulfide conjugate is not clear, sequestration of tapasin by PDI, in turn, might affect the levels of the ERp57–tapasin conjugate in the peptide-loading complex.

Given the number of intra- and intermolecular disulfide bonds that exist among the components of the peptide-loading complex, thiol-based redox regulation is thought to play a certain role in loading peptides onto MHC class I molecules. The complete oxidation of MHC class I molecules is essential for optimal peptide loading (207, 261). The MHC class I molecule has two disulfide bonds in the α_2 and α_3 domains (30). The disulfide bond in the α_2 domain is of particular interest because this disulfide bond is located within the peptide-binding groove (30). The α_2 disulfide bond might directly affect peptide binding; conversely, peptide binding could protect the disulfide bond and make it less accessible to reduction. As expected, disruption of the α_2 domain disulfide bridge of HLA-A0201, by mutating cysteine 101 to a serine (C101S) or cysteine 164 to alanine (C164A), decreased the efficiency of peptide loading and the level of MHC class I surface expression (303). Because the redox state of the α_2 domain is a determinant of proper assembly of the MHC class I complex, determining which proteins regulate the redox state of the α_2 disulfide bond is crucial. The presence of ERp57 in the peptide-loading complex initially suggested that ERp57 might be involved in redox regulation of the α_2 disulfide bond. However, in ERp57-deficient mice, the redox state of MHC class I molecules was not affected (89). RNA interference-mediated knockdown of ERp57 in a cultured cell line did not affect the peptide loading and redox state of MHC class I molecules (320). Thus, direct involvement of ERp57 in regulation of the α_2 disulfide bond appears to be unlikely.

B. Function of the ERp57–tapasin conjugate

Many functions were proposed for the ERp57–tapasin conjugate, after its discovery within the peptide-loading complex, in MHC class I peptide loading. Because the cysteine at position 95 of tapasin is involved in formation of an intermolecular disulfide bond with ERp57 (61), a C95A tapasin mutant was used to examine the functional role of the ERp57–tapasin conjugate in antigen processing. Cresswell and colleagues (61) analyzed the assembly of MHC class I molecules in tapasin-negative .220.B44 cells that were transiently expressing either wild-type or the C95A mutant tapasin. Disruption of the tapasin–ERp57 conjugate with the C95A mutant impaired the assembly of MHC class I molecules. In cells expressing the tapasin C95A mutant, the MHC class I heavy chain was partially reduced within the peptide-loading complex, and the resulting MHC class I–β_2m heterodimers were inefficiently loaded with peptides (61).

These data indicate that the ERp57–tapasin conjugate is critical for proper MHC class I assembly, but the mechanism underlying this function is unclear. However, recent reports indicate that in the absence of tapasin, ERp57 reduced the disulfide bond in the α_2 domain of the MHC class I molecules. Thus, the covalent bond between tapasin and ERp57 appears to sequester ERp57 and prevents the reductase activity of ERp57 from acting against the α_2 disulfide bond of oxidized class I molecules, which is necessary for maintaining the peptide-binding groove in a peptide-receptive form (138). In tapasin-deficient cells or cells expressing the C95A mutant, the amount of the ERp57–MHC class I heavy-chain conjugate increased in comparison to cells expressing normal tapasin (138). Therefore, ERp57-mediated reduction of the MHC class I heavy chain appears to be dependent on tapasin. Individual

MHC class I alleles show different dependencies on tapasin for ERp57-mediated reduction. HLA-B4402 is tapasin dependent for optimal peptide loading, whereas HLA-B4405 can be efficiently loaded with peptides even in the absence of tapasin (312). Further, in cells expressing the C95A tapasin mutant, HLA-B4402 is in the reduced form, whereas HLA-B4405 is primarily oxidized (138). This tapasin dependence also appears to have a role in MHC class I folding, because the ERp57–MHC class I heavy-chain conjugate associates with calreticulin, whereas the ERp57–tapasin conjugate inhibits the ERp57–calreticulin-based disulfide exchange (138). Notably, the reductase activity of ERp57 is seen only in the absence of tapasin (138), so the physiological significance of these observations in normal cells requires further investigation. A recent study by Cresswell's group (221) shows that in the absence of ERp57, the a and a' domain redox activity in peptide loading occurs normally, arguing that the redox activity of ERp57 is not essential for its function in peptide loading. Therefore, in MHC class I peptide loading, other features of ERp57 might account for its significance.

One of the proposed functions of tapasin is to stabilize MHC class I molecules and optimize, quantitatively and qualitatively, the peptide repertoire that is loaded into the MHC class I molecules (312). Another clue for the function of the ERp57–tapasin conjugate has come from a reconstitution experiment in which the peptide-loading subcomplex from tapasin-negative .220 cell extracts was reconstituted with recombinant soluble tapasin, the tapasin–ERp57 conjugate, or other purified components of the peptide-loading complex (304). In this reconstitution system, recombinant tapasin–ERp57 heterodimers play a role in stabilizing empty MHC class I molecules, in facilitating peptide loading, and in editing the repertoire of the bound peptides (304). When either the wild-type or C60A ERp57 trapping mutant was used, the activities of the tapasin-ERp57 conjugate were almost identical (304). This result suggests that ERp57 serves a structural role that is required for the function of tapasin, whereas the catalytic activity of the a domain in ERp57 is dispensable for peptide loading.

C. Function of PDI in the peptide-loading complex

The importance of redox regulation in the peptide-loading complex was further underscored by recent identification of PDI as a novel component of the peptide-loading complex (218). The presence of two oxidoreductases, ERp57 and PDI, in the peptide-loading complex indicates that redox regulation is more complicated than previously thought. PDI has been identified in the TAP complex by co-immunoprecipitation and mass spectrometry analysis (Fig. 10), and because PDI functions include disulfide bond oxidation, reduction, and isomerization, PDI involvement is speculated in the redox regulation of the peptide-loading complex (67).

Knockdown of PDI by RNA interference reduces the surface expression of fully assembled MHC class I molecules, whereas the surface levels of β_2m-free MHC class I heavy chain increase (218). This finding suggests that the assembly of MHC class I is impaired in the absence of PDI. In addition, knockdown of PDI delays the maturation kinetics of MHC class I molecules and interferes with optimal peptide loading onto MHC class I molecules (218), demonstrated by endoglycosidase H analysis and thermostability assay, respectively. The endoglycosidase H analysis makes it possible to estimate the ER exit rate of glycoproteins, whereas the thermostability assay correlates thermostability with the affinity of MHC class I for its peptide cargo (312). Interestingly, the maturation rate and thermostability of MHC class I molecules were restored by transient expression of an a' domain deletion mutant of PDI (PDI-abb'). However, ectopic expression of either a catalytic site mutant or peptide-binding–site mutant of PDI did not restore the maturation rate or thermostability of MHC class I molecules. These results indicate that both the catalytic function and peptide-chaperoning functions of PDI are required for the proper assembly of MHC class I complexes. Further, PDI depletion increased the pool of reduced MHC class I molecules (218). The observation that the peptide-binding–site mutant PDI did not restore the redox state of MHC class I molecules suggests that the supply of antigenic peptides affects the redox state of MHC class I molecules. Indeed, as seen in PDI-depleted cells, in cells in which viral TAP inhibitors block the delivery of peptides into the ER lumen, the reduced form of MHC class I molecules accumulates in the peptide-loading complex (218). These results demonstrate that peptide loading is closely linked with the redox regulation of MHC class I molecules in the peptide-loading complex. Furthermore, our group detected a disulfide intermediate between MHC class I molecules and PDI. When the two cysteines in the α_2 domain of the MHC class I heavy chain are mutated, this disulfide intermediate is abolished (218), indicating that PDI is involved in the formation of the disulfide bond within the peptide-binding groove of the α_2 domain. Because the disulfide bond in the α_2 domain is buried when MHC class I molecules are loaded with peptides (30), optimal peptide loading is expected to correlate with the oxidation state of the α_2 domain of the MHC class I molecule.

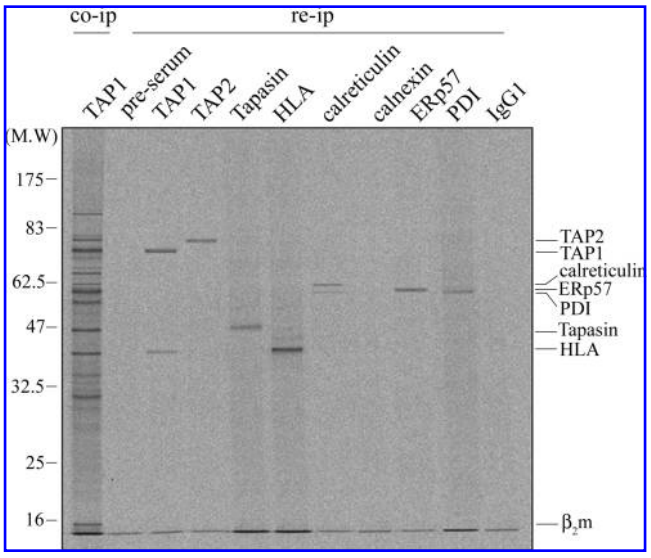


FIG. 10. Association of PDI with components of the peptide-loading complex. HeLa cells were radiolabeled, lysed in 1% digitonin-10 m*M* NEM and immunoprecipitated with TAP1 antibody. Eluted samples were reprecipitated with the indicated antibodies. This result shows the association of PDI with the peptide-loading complex. Adapted from reference 218, with permission.

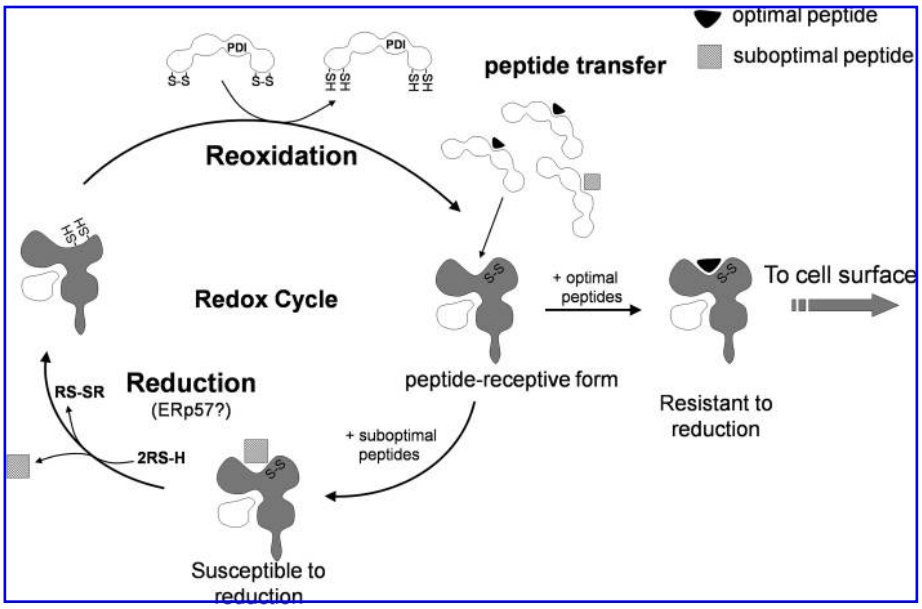


FIG. 11. Model for the function of PDI in peptide loading of MHC class I molecules. MHC class I molecules use the PDI-mediated redox cycle for optimal peptide loading. Oxidizing the α_2 domain disulfide bond by PDI maintains the peptide-receptive state of MHC class I molecules in the peptide-loading complex. PDI transfers peptides to the fully oxidized MHC class I molecules. MHC class I molecules loaded with an optimally structured peptide are resistant to reduction and can exit the ER. However, suboptimal peptide loading cannot prevent MHC class I molecules from undergoing reduction of the α_2 domain disulfide bond, causing the suboptimal peptide to disassociate from the reduced MHC class I molecules. Reduced MHC class I molecules are reoxidized by PDI to reinitiate the cycle.

D. The model for redox-regulated peptide editing

On the basis of the preceding results and the structural properties of MHC class I molecules, the likely primary functions of PDI in peptide loading are maintaining the disulfide bond within the peptide-binding groove of the α_2 domain and facilitating the transfer of a peptide to the MHC class I molecules. We propose a hypothetical model for the role of PDI, with respect to the redox state of the MHC class I molecules, in selecting optimal peptides (Fig. 11). In the peptide-loading complex, the redox state of the MHC class I molecule is in dynamic equilibrium between reduced and oxidized states. After oxidation of the α_2 disulfide bond by PDI, the peptide-binding groove of the MHC class I molecule has the proper conformation for peptide loading, and PDI then transfers peptides to MHC class I molecules. MHC class I molecules loaded with an optimally structured peptide are resistant to reduction because of a shielding of the disulfide bond by the peptide, whereas suboptimal peptide-loaded or empty MHC class I molecules are susceptible to reduction. This reduction may be catalyzed by ERp57. Reduced MHC class I molecules can be reoxidized by PDI to undergo a successive round of peptide loading and potential addition of an optimally structured peptide.

An alternative, but not mutually exclusive, hypothesis to describe the functions of PDI in peptide editing is the "Venus flytrap" model (68, 311). This model proposes that MHC class I molecules exist in two conformations, "open" and "closed," with open MHC class I molecules more receptive to peptide loading than closed MHC class I molecules. Oxidation of the α_2 disulfide bond of the MHC class I heavy chain by PDI may transform the peptide-binding groove from the closed to the open conformation. Loading of the peptide into the groove then changes the conformation from open to closed. If a suboptimal peptide is loaded, the reopening of the closed MHC class I molecule would be necessary for optimal peptide loading. In this scenario, ERp57 and PDI might cooperate to reduce and reoxidize the α_2 disulfide bond, resulting in the selection of high-affinity peptides for loading into the MHC class I complex.

VII. Regulation of Substrate Binding Affinity by the Redox Cycle

A. A potential role for PDI as a peptide carrier

One of the key questions in antigen processing is how peptides are delivered from TAP to the peptide-binding groove of the MHC class I molecules. This process occurs in the highly proteolytic environment of the ER lumen, which is unfavorable for delivery of peptides from TAP to MHC class I molecules. In the ER, free peptides are unstable (235), yet only 1 in 80,000 peptides is expected to have the right structure for the MHC class I peptide-binding groove (68, 314). Thus, instead of simple diffusion, delivery of peptides from TAP to MHC class I molecules might occur *via* molecular chaperones that protect bound peptides from degradation, allowing them to survive longer in the ER. Chaperones that bind TAP-translocated peptides have been identified (264, 266). Among these chaperones, PDI is the best candidate to play a role in peptide delivery for several reasons. First, PDI is a major protein that is bound to TAP-translocated peptides in the ER (151, 264). By using radioactive peptides with a photoreactive group, Spee *et al.* (264) identified PDI as one of the peptide-binding proteins in the lumen of the ER (264).

With a similar approach, Lammert *et al.* (151) also showed that TAP-translocated peptides can be efficiently conjugated to PDI. PDI is the only ER protein that is labeled by the photoreactive peptides (Fig. 12). Binding of TAP-translocated peptides to PDI appears to be specific, because their association can be inhibited by β-estradiol (151). The β-estradiol binds the substrate binding sites of PDI (287), thereby

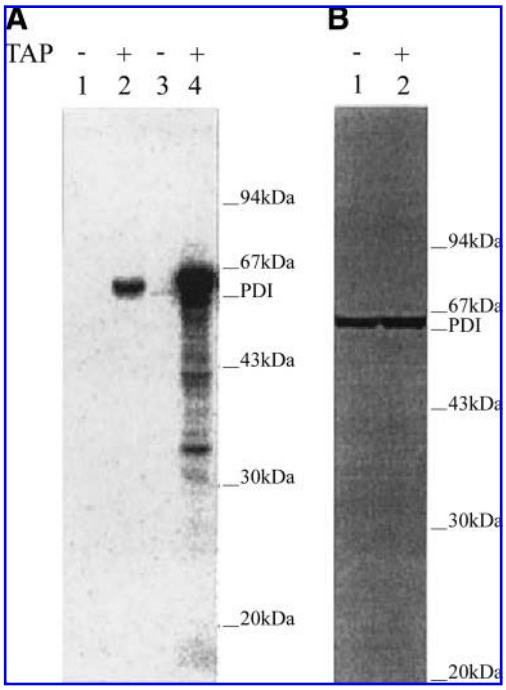


FIG. 12. PDI is the dominant acceptor for TAP-translocated peptides. (**A**) Immunoprecipitation of PDI extracted from T2 (TAP^-) cells and T2 cells expressing both recombinant TAP1 and TAP2 (TAP^+) after translocation and cross-linking of radioiodinated peptides. Lanes 1 and 2, nef7B peptide; lanes 3 and 4, TNKTRIDGQ(Tpa)Y; lanes 1 and 3, T2 (TAP^-); and lanes 2 and 4, T2 (TAP^+). (**B**) Western blot of cellular extracts from T2 (TAP^-) (lane 1) and T2 (TAP^+) (lane 2) cells showing PDI. Adapted from reference 151, with permission.

inhibiting binding of peptide to PDI. These data suggest that PDI might be a major acceptor for antigenic peptides translocated by TAP. Second, PDI associates with the peptide-loading complex (Fig. 10), and, unlike ERp57, PDI possesses a binding site for small peptides (120, 218, 226). Third, mutation of this small peptide-binding site in the b′ domain of PDI interferes with optimal peptide loading into MHC class I molecules (218).

B. Regulation of substrate binding and release by chaperones

Assisting protein folding is a function of chaperones that relies on their unique ability to bind and release their substrates repeatedly, a cycle that is controlled in various ways. Heat-shock protein 33 (Hsp33) is a chaperone, the activity of which is regulated by its redox state (211). Under normal conditions, fully reduced Hsp33 exists as a monomer, with all four cysteines involved in zinc coordination and low affinity for its folding substrate (230). However, under oxidative stress, Hsp33 is rapidly activated as a potent molecular chaperone by making intramolecular disulfide bonds (125). The four cysteines of Hsp33 release the zinc ion and quickly form two intramolecular disulfide bonds (22, 125). The formation of the disulfide bonds and release of zinc induces generation of the Hsp33 dimer that is competent to bind substrates (94, 119) (Fig. 13).

Another example in which redox state is related to substrate binding is the anti-σ factor, RsrA of *Streptomyces coelicolor* (134). Sigma factor σ^R is required for the induction of the reductase-thioredoxin operon (*trxBA*) of *S. coelicolor* under several oxidizing conditions. On transient induction of *trxBA* by diamide treatment, σ^R causes the expression of the thioredoxin system in response to cytoplasmic disulfide bond formation (212). However, as σ^R has no cysteines, σ^R needs another component that can sense the redox environment. Roe's group (135) showed that the δ^R-specific anti-sigma factor activity of RsrA is directly regulated by reversible disulfide bond formation. The disulfide bond formation leads to the release of σ^R, which subsequently activates the transcription of its target genes.

In contrast to Hsp33 and RsrA, which rely on redox regulation for their substrate binding and release, Hsp70 uses

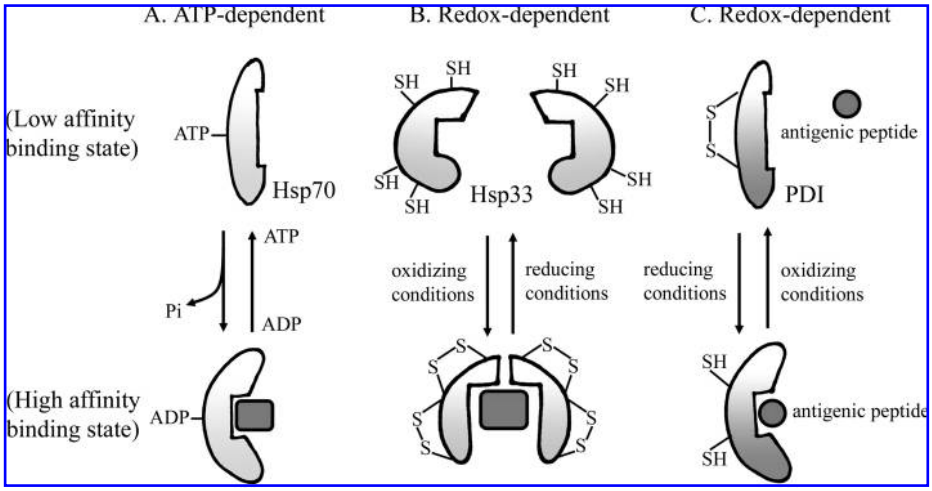


FIG. 13. The cycle of molecular chaperones between low-affinity and high-affinity substrate-binding states. Several different ways exist to regulate chaperone activity. Hsp33 or PDI are redox-regulated chaperones that use reversible disulfide bond formation to regulate their substrate-binding affinity. (**A**) A representative of ATP-dependent chaperones, Hsp70 has a binding cycle that is regulated by ATP (35). In the case of Hsp70, ATP hydrolysis accelerates substrate binding. (**B**) A similar type of regulation can be found in the redox-dependent chaperone, Hsp33. Structural changes of redox-dependent chaperones are induced by oxidizing or reducing disulfide bonds and tightly regulate the substrate-binding affinity of these chaperones. (**C**) In the case of PDI, a change in the redox state induces conformational changes in the protein. Structural changes in PDI induced by the redox environment regulate its substrate binding, including antigenic peptide binding. The position of thiol groups and disulfide bonds is for illustration only.

nucleotide exchange to operate its chaperone function. On binding of ATP to Hsp70, Hsp70 has an open peptide-binding pocket. However, the interaction between Hsp70 and the substrate peptide is weak in this conformation, allowing peptides to enter and leave the binding pocket easily. Once ATP is hydrolyzed, the peptide-binding pocket is closed, and peptide is bound strongly. Exchange of the ADP for a new ATP molecule induces the release of peptide (35).

C. Redox regulation of peptide binding and release by PDI

If PDI is a major peptide receptor or peptide carrier, one must explain how PDI could regulate peptide binding and release. PDI is the only member of protein thiol-disulfide oxidoreductases capable of catalyzing both the oxidation and reduction of protein disulfides under physiologic conditions (200). PDI has two redox-active CXXC motifs and acts as an oxidoreductase, isomerase, and ATP-independent chaperone. Furthermore, the particular activity that PDI exhibits is related to the redox state of the protein. For example, when the cysteines of PDI are reduced, it acts as isomerase, reshuffling already formed disulfide bonds in ER proteins (200). Therefore, we propose that PDI, similar to Hsp33, binds and releases peptide in a redox-dependent manner (Fig. 13C), thereby serving as an antigenic peptide carrier in the ER. The reduced form of PDI binds the peptide at the point of entry into the ER, followed by delivery to the MHC class I molecules. Oxidation of PDI in proximity to MHC class I molecules induces the release of peptide and subsequent peptide loading onto MHC class I molecules. This model predicts that different redox microenvironments would exist outside and inside the peptide-loading complex (Fig. 13C). Consistent with the notion that binding and release of substrate can be regulated by the redox state of PDI, Rapoport and co-workers (286) demonstrated that PDI disassembles cholera toxin in a redox-dependent manner, indicating that PDI is a redox-regulated chaperone. Cholera toxin is produced by the bacterium *Vibrio cholerae* and consists of A and B subunits. The A subunit (A_1), the catalytically active component, is transported into the cytosol of target cells after its release from the B subunits (251). To release the A_1 chain, disulfide bridges of A_1 must be reduced, as only an unfolded A_1 chain can be transported through the Sec61p channel. PDI is responsible for the disassembly and unfolding of the toxin A_1 chain. Surprisingly, in a reduced state, PDI interacts with toxin and unfolds the toxin, whereas in the oxidized state, PDI releases the A_1 chain substrate. These results suggest that the structural conformation of PDI might change, depending on its redox state. Interestingly, this redox-regulated binding cycle of PDI is similar to ATP-binding cycle of Hsp70. It is remarkable that PDI and Hsp70 have evolved two different reactions, an ATP-independent redox reaction and ATP hydrolysis, to regulate the seemingly similar chaperone function of substrate binding and release.

It is not clear whether redox-dependent binding and release of substrates by PDI can be generalized to other substrates. Bulleid's group (165) showed that binding of the C-propeptide of procollagen, a PDI substrate with which it forms a transient interaction during its folding pathway, is not regulated by the redox state of PDI. Hence, the ability of PDI to act as a redox-dependent chaperone in binding and release of substrates might be dependent on the substrates. Much work is needed to understand the mechanisms that govern the binding and release of substrates by PDI.

VIII. Redox Regulation of MHC Class I Disassembly and ER Exit of Peptide-Loaded MHC Class I Molecules

A. Export of proteins from the ER

Once secretory proteins are synthesized and folded in the ER, they exit the ER either by bulk flow or by a selective transport mechanism (101, 307). Transport between the membrane compartments of the secretory pathway is mediated by membrane vesicles. Vesicles are formed by a budding mechanism involving coat proteins that capture specific cargo molecules into coated vesicle intermediates. These vesicle intermediates form at a specific site of the ER, the ER exit site (184), and the budding of COPII-coated vesicles occurs at this site. COPII-coated vesicles mediate forward transport from the ER through the Golgi apparatus. COPII comprises at least five components, Sar1p, Sec23p, Sec13, Sec31, and Sec24p, and COPII vesicles are made by the concerted action of these subunits of the coat (176). The COPII-coat machinery forces curvature of the ER membrane to form a coated vesicle (157). When COPII-coated vesicles move and fuse with their targets, COPII-coat proteins are disassembled, and the cargo molecules are discharged into the target compartment.

B. Redox regulation of MHC class I disassembly

MHC class I molecules exit from the ER by selective transport within COPII-coated vesicle (265). After peptide loading and quality control, MHC class I molecules are exported from the ER in a process that can be divided into two steps: release of peptide-loaded MHC class I molecules from the peptide-loading complex and COPII-mediated vesicle budding (272).

Optimal peptide loading onto the MHC class I complex is thought to be sufficient for its release from the peptide-loading complex and subsequent export from the ER (213). Howard and co-workers (142) reported that TAP is also involved in the dissociation of MHC class I molecules from the peptide-loading complex. Howard's group observed that disruption of the nucleotide-binding site of TAP blocks the release of properly loaded MHC class I molecules from the peptide-loading complex, yet the TAP mutant formed normal loading complexes. Further, in cell lysates prepared with digitonin, the addition of peptides that bind MHC class I molecules allowed the dissociation of peptide-loading complexes made with wild-type TAP proteins; however, complexes made with mutant TAP proteins could not be dissociated. These data indicate that dissociation of MHC class I molecules from the peptide-loading complex requires conformational signals transmitted from TAP (142). Whether dissociation of peptide-loaded MHC class I molecules from TAP is sufficient for their export from the ER has yet to be determined.

The mechanisms for the disassembly of the peptide-loading complex and release of the MHC class I complex after peptide loading remain uncharacterized. Given that the tapasin–ERp57 conjugate is quite stable and functions to recruit the MHC class I heavy chain into the peptide-loading complex (304), it is of particular interest to determine whether the dissociation of the tapasin–ERp57 disulfide-linked conjugate

precedes release of peptide-loaded MHC class I molecules from the peptide-loading complex and their subsequent ER egress.

A potential regulatory role for the tapasin–ERp57 conjugate in the ER exit of MHC class I molecules has been implicated in several studies. In ERp57-negative cells, optimal peptide loading of MHC class I molecules is impaired, and their export rate from the ER is accelerated by about twofold (89). Similar to the phenotype in ERp57-negative cells, in tapasin-negative cells, most MHC class I molecules fail to acquire high-affinity peptides, and these MHC class I heavy-chain–β_2m heterodimers efficiently exit the ER (93). By using insect cells as an assay system to reconstitute MHC class I antigen presentation, Fruh and co-workers (249) demonstrated that tapasin increases MHC class I peptide loading by retaining empty, but not peptide-loaded, MHC class I molecules in the ER. Unlike most HLA-A and HLA-B alleles, HLA-C alleles associate poorly with β_2m (197). These HLA-C alleles are exported more rapidly from the ER than are the HLA-A and HLA-B alleles (196). The independent recognition of the activity of tapasin in bridging TAP to MHC class I heavy-chain–β_2m heterodimers (206) and retaining MHC class I molecules in the ER reflects the critical function of the ERp57–tapasin conjugate in retaining empty MHC class I molecules in the ER until they are loaded with optimal peptides. These observations also argue that impaired function of the tapasin–ERp57 conjugate results in a loss of stringency in quality control of MHC class I molecules.

Recently, we made interesting observations that could provide insight into how the formation and dissociation of the tapasin–ERp57 conjugate are regulated to control the ER exit of peptide-loaded MHC class I molecules. In cells overexpressing wild-type PDI, retention of MHC class I molecules in the ER is accompanied by concomitant accumulation of the tapasin–ERp57 conjugate (unpublished observation). Unexpectedly, the same phenomena were observed in PDI- or peptide-depleted cells. Ectopic expression of the peptide binding-site mutant of PDI also leads to delayed exit of MHC class I molecules from the ER and accumulation of the tapasin–ERp57 conjugate.

Further, we noted that PDI bound to peptides has a higher affinity for the tapasin–ERp57 conjugate than does "empty" PDI (unpublished observations). These results are consistent with the model of PDI acting as a peptide-dependent molecular switch that regulates the dissociation of the tapasin–ERp57 disulfide conjugate and controls the ER export of MHC class I molecules. We thus speculate that on peptide-binding, PDI undergoes a conformational change that increases its affinity for the tapasin–ERp57 conjugate in the peptide-loading complex (Fig. 14). The subsequent binding of the peptide-bound PDI to the tapasin–ERp57 conjugate distorts the structural features of the tapasin–ERp57 heterodimer, such that the feature of tapasin that masks the ERp57 is altered. Unmasking of ERp57 activates an escape pathway that results in the disassociation of the tapasin–ERp57 conjugate (Fig. 15). In PDI-overexpressing or peptide-depleted cells, the accumulation of the tapasin–ERp57 conjugate and delayed ER exit of MHC class I molecules are likely caused by an increase in the "empty" PDI pool that is unable to bind to the tapasin–ERp57 conjugate and, therefore, cannot activate the escape pathway. Collectively, our data suggest that PDI-mediated reduction of the tapasin–ERp57 disulfide conjugate is necessary for the disassembly of the peptide-loading complex and subsequent ER export of MHC class I molecules.

C. ER exit of the MHC class I–peptide complex

The dissociation of the tapasin–ERp57 disulfide conjugate seemingly precedes the release of peptide-loaded MHC I molecules from the peptide-loading complex and their export from the ER; nevertheless, whether the mechanism by which the peptide-loading complex is disassembled is directly associated to the ER exit of MHC class I molecules is uncharacterized. The direct relevance between these events awaits further investigation.

The export of peptide-loaded MHC class I molecules from the ER occurs by their binding to unidentified cargo receptors, mobilizing to the ER exit site, and budding of COPII vesicles (265). Before they exit the ER, properly folded and peptide-loaded MHC class I molecules form clusters that are distinct from the clusters formed by unassembled or misfolded MHC I molecules (224).

After the release of peptide-loaded MHC class I molecules from the peptide-loading complex, they associate with calnexin and persist in the ER for a short time (170, 273). The physiologic function of this association remains unknown, although this interaction may be another step that determines ER exit of MHC I molecules (273). During this time, peptide-loaded MHC class I molecules might undergo further regulation before their incorporation into COPII vesicles. For example, ER exit of some proteins requires posttranslational modification. In the case of lipoprotein receptor–related protein 6, palmitoylation and ubiquitination regulate ER exit (2). Similarly, the cysteine residues in the cytoplasmic tail of MHC class I molecules are palmitoylated, and mutations in these cysteine residues impair the egress of HLA-B7 from the ER (99). Although the cysteine residues in the cytoplasmic tail are not conserved among all MHC class I alleles, this result suggests that palmitoylation might comprise parts of ER export signals for some MHC class I molecules. Ubiquitination of MHC class I molecules has also been reported; however, these studies relate degradation of unfolded and misfolded MHC I molecules followed by dislocation to cytoplasm and degradation by proteasomes but not true ER exit (38, 256).

MHC class I molecules are thought to be recruited to ER exit sites by B-cell receptor–associated protein 31 (BAP31). First, MHC class I molecules associate with BAP31 (150, 214, 265). In the absence of BAP31, MHC class I molecules fail to localize with mSec31, a component of the COPII coat protein complex, and the transport of MHC class I molecules from the ER to the Golgi apparatus is delayed (214). Similarly, overexpression of BAP31 accelerates the export of MHC class I molecules to the cell surface (150). Data from endo H analysis and thermostability assays suggest that peptide loading and glycosylation of MHC class I molecules occur normally in the same cells (150). Thus, the acceleration of MHC class I export in cells overexpressing BAP31 may be caused by change in another export-regulatory system rather than irrelevant folding of MHC I molecules. The function of BAP31 as the MHC class I cargo receptor might be redundant because knockdown of BAP31 did not affect the surface expression of MHC class I molecules (150). Identification of additional MHC class I cargo receptors may contribute to our under-

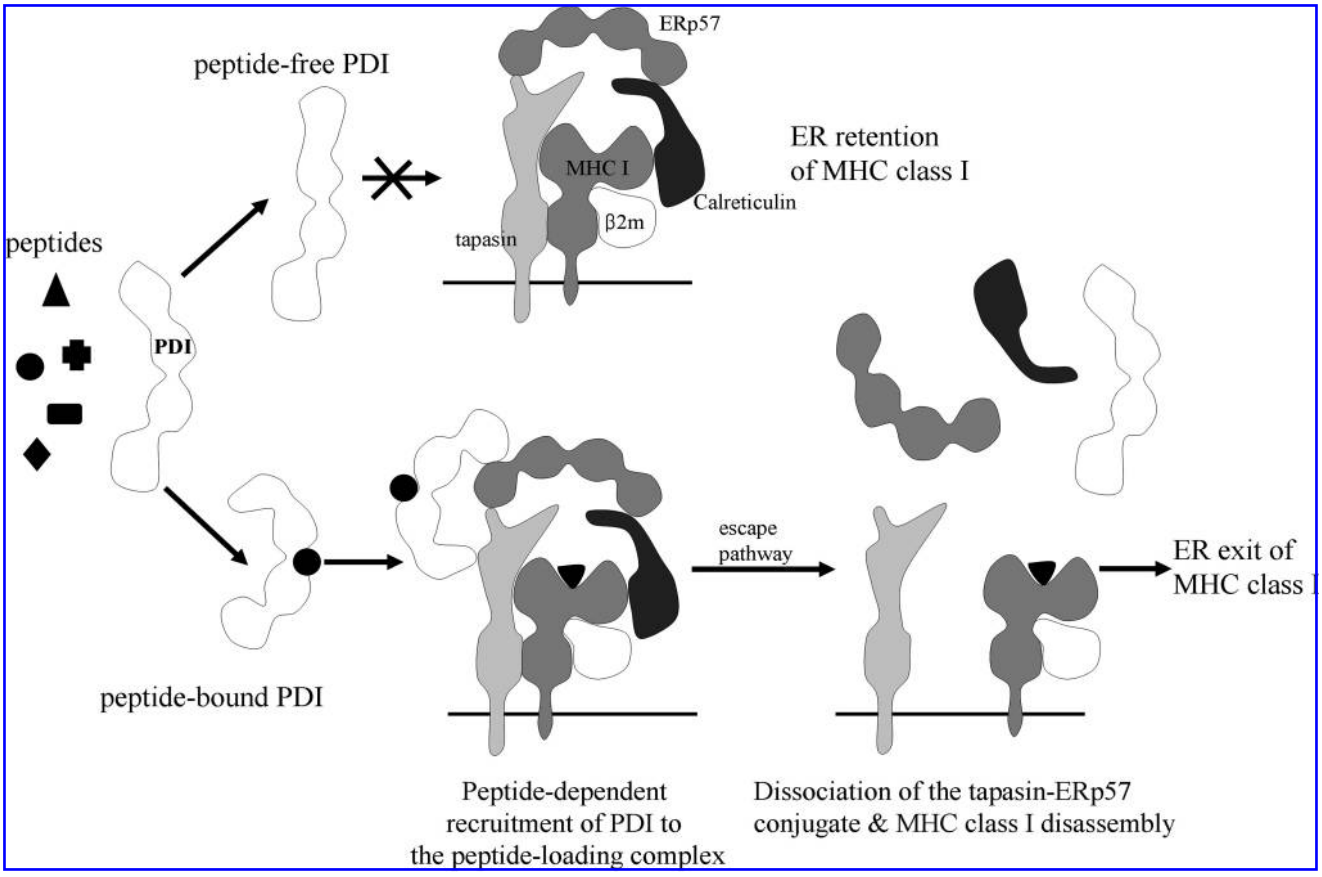


FIG. 14. Model for PDI as a peptide-dependent molecular switch. In the peptide-bound state, PDI is recruited to the peptide-loading complex and binds to tapasin and ERp57. PDI induces conformational changes in tapasin and, in turn, activates the escape pathway of ERp57. This activation initiates the disassembly of the peptide-loading complex and release of the complete MHC class I complexes. In the absence of peptides, peptide-free PDI is not competent to bind to tapasin or ERp57. The escape pathway remains inactivated. As a result, the MHC class I molecules cannot be released from the peptide-loading complex.

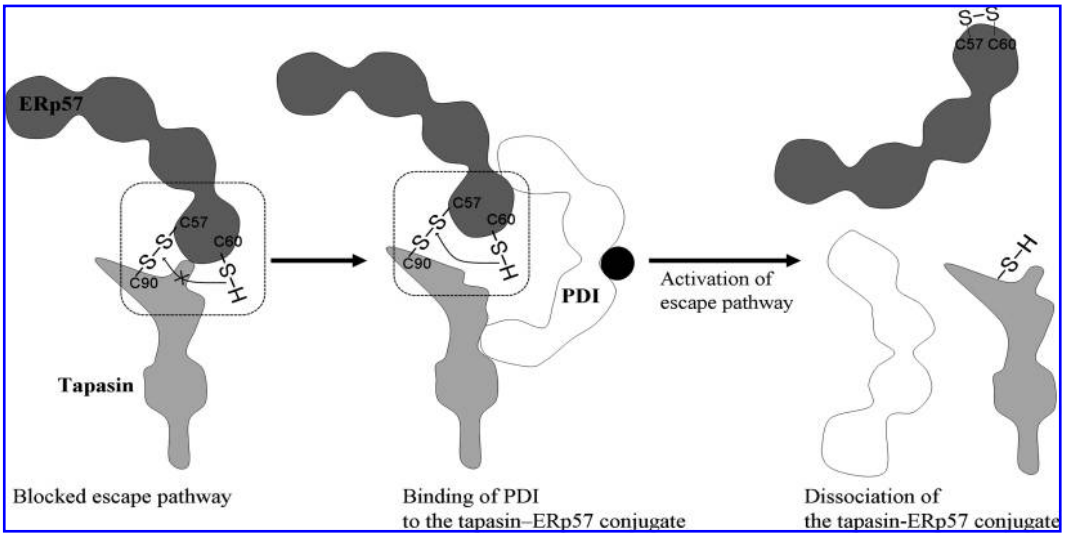


FIG. 15. Model for dissociation of the tapasin–ERp57 disulfide conjugate by PDI. Noncovalent interaction between tapasin and ERp57 prevents the free thiol group of ERp57 cys^{60} from attacking the inter-disulfide bond between tapasin and ERp57, thereby inactivating the escape pathway of ERp57 (222). PDI binds to the tapasin–ERp57 conjugate and distorts the conformation of tapasin, unmasking the free thiol group of ERp57 and activating the escape pathway. Escape of ERp57 from tapasin involves the formation of a cys^{57}-cys^{60} intramolecular disulfide bond, allowing the tapasin–ERp57 conjugate to be dissociated.

standing of the quality control of proteins and antigen processing.

IX. Role of Redox Regulation of MHC Class I–Restricted Antigen Processing in Disease

Regulation of the intracellular redox environment is critical for cell viability (76), cell activation (13), and cell proliferation (174). Various environmental factors, such as UV (59, 60, 246, 247), ionizing radiation (291), nitric oxide (152), heavy metals (195), and viral infection induce the generation of reactive oxygen species (ROS). In particular, some virus infections, such as hepatitis C virus and human immunodeficiency virus, are associated with elevated levels of ROS and reactive nitrogen species and decreased antioxidant levels in patients (21, 45, 124, 167, 238, 275). With several different viruses, after infection, oxidative stress occurs (9, 84, 204) and enhances viral replication. For example, HIV replication is enhanced under oxidative conditions (20, 194, 267). ROS also activates nuclear factor kappa B (NF-κB), which is required for HIV replication (159, 194).

During the last decade, various molecules involved in redox regulation have been discovered, and their functional roles in diseases have been reported. In particular, imbalance of the reduction and oxidation states is implicated in various diseases, such as cancer (228), viral infection (173, 252, 253), ischemia/reperfusion injury (209), cardiac conditions (48), aging (121), premature birth (53, 54, 71), and newborn physiology (53). For MHC class I molecules, redox regulation, which affects their folding or peptide loading, was recently noticed (221, 243). ERp57, tapasin, and PDI are involved in redox regulation of MHC class I molecules (138, 218).

MHC class I–restricted antigen processing and presentation is imperative for the induction of $CD8^+$ T-cell immune responses against infected and cancerous cells. Viruses and tumors have evolved various mechanisms to counteract this powerful host immune response. Human cytomegalovirus (HCMV), a herpesvirus, has >70% prevalence in the population worldwide. HCMV causes a serious disease in immunocompromised and immunosuppressed adults and is fatal to patients with HIV, organ-transplant recipients, or neonates (11, 118). After the primary infection is resolved, HCMV establishes a persistent infection, which is facilitated by viral immune evasion (241). Immune evasion strategies of HCMV are primarily related to antigen presentation (161, 308). HCMV encodes several gene products from the unique short region, and each protein is independently able to inhibit MHC class I–restricted antigen presentation (6). The HCMV gene product US2 destabilizes MHC class I heavy chains (131), and US3 impairs transport and maturation of MHC class I heavy chains (6, 132). US6 inhibits peptide translocation by TAP (7, 113). US11 dislocates MHC class I heavy chains from the ER to the cytosol (309).

Our group recently reported that the HCMV gene product US3 targets the degradation of PDI to facilitate the immune evasion of the virus (218). Because PDI has several functions, early oxidative folding of MHC class I molecules, optimal peptide loading, and ER exit of peptide-loaded MHC class I molecules, in the redox regulation of antigen processing, the degradation of PDI by US3 inhibits MHC class I–mediated antigen processing and presentation, allowing the virus-infected cell to evade the $CD8^+$ T-cell response. This represents a novel example of a viral protein that, for immune evasion, disrupts the regulation of the redox network involved in antigen processing, emphasizing the importance of redox regulation in antigen processing. It would be of considerable interest to investigate whether other viruses can also exploit the redox network of the antigen-processing machinery to evade immune response.

Misfolding of MHC class I proteins by aberrant intermolecular disulfide bond formation has been linked to autoimmune diseases. The MHC class I allele HLA-B27 is associated with development of the inflammatory arthritic disease, ankylosing spondylitis (231). The HLA-B27 allele is expressed by about 95% of patients with ankylosing spondylitis, and although the role of HLA-B27 in this disease is not clear, the available evidence suggests that oxidative misfolding of MHC class I might be a cause of ankylosing spondylitis. HLA-B27 contains an unusual cysteine residue at position 67, and this residue is involved in an intermolecular disulfide bond that results in HLA-B27 homodimers instead of HLA-B27–β_2m heterodimers (12, 14, 17, 50). The HLA-B27 homodimers are displayed on the cell surface (28). An unusual form of surface-expressed B27 that is loaded with unconventionally long peptides and lacks β_2m has been described (168). The HLA-B27 homodimers are recognized by various immune cell receptors (12, 147), which might induce the aberrant immune response.

Although these results suggest that aberrant formation of an intermolecular disulfide bond through cysteine 67 in HLA-B27 may be important in the pathogenesis of ankylosing spondylitis, compelling evidence is lacking. Rather, a study in transgenic rats indicates that the phenotype of HLA-B27–associated inflammatory disease is, at most, modestly affected by the cysteine-to-serine mutation at position 67 (C67S) (285). Analysis of C67S mutant spleen cells derived from transgenic rats reveals the presence of disulfide-linked homodimers at the cell surface, indicating that formation of homodimers involves other cysteine residues in B27 (285). These data raise the possibility that the peptide-binding specificity of B27, but not formation of homodimers, may be responsible for disease pathogenesis.

X. Conclusions and Perspectives

Antigen processing is the first step in the induction of T cell–mediated adaptive immune responses. The assembly of MHC class I–peptide complexes is highly regulated, with redox regulation playing a critical role. In the early stage of MHC class I assembly, the correct folding of newly synthesized MHC class I heavy chains requires intradisulfide bond formation that is catalyzed by the ER oxidoreductase, ERp57. The tapasin–ERp57 disulfide conjugate is essential for recruiting MHC class I–β_2m heterodimers into the peptide-loading complex and retaining them within the ER until they become loaded with high-affinity peptides. PDI catalyzes the disulfide bond formation within the MHC peptide-binding groove, thereby facilitating optimal peptide selection by the MHC class I molecules. In addition, PDI controls MHC class I disassembly and subsequent exit of peptide-filled MHC class I molecules from the ER by regulating the dissociation of the tapasin–ERp57 conjugates in a peptide-dependent manner. Collectively, these thiol-based redox reactions ensure that only correctly assembled MHC class I–peptide complexes are

transported to the cell surface. Failure of these redox regulatory processes results in immune deficiency or abnormal immune responses.

Over the past few years, recent advances in redox biology and cell biology have revealed that thiol-based redox regulation features prominently in antigen processing. The discovery of a link between antigen processing and redox regulation was unexpected. Because signaling pathways that involve redox regulation are rich sources of validated drug targets, these newly described mechanisms of antigen processing represent opportunities for identifying and developing new drugs. However, many questions regarding the mechanisms of regulation remain unanswered. For example, similar redox regulatory principles might apply to MHC class II–restricted antigen processing? Compared with MHC class I, much less is known about the importance of redox regulation in MHC class II antigen presentation. Considering that MHC class II α- and β-chains contain conserved cysteine residues, the folding and assembly of MHC class II may undergo a quality control involving redox regulation in the ER. The recent studies by Cresswell and his co-workers (19) show that redox regulation of MHC class II antigen processing can occur in the intracellular compartments beyond the ER. γ-IFN–inducible lysosomal thiol reductase (GILT) is an oxidoreductase containing a thioredoxin-like CXXC motif that is present in the MHC class II loading compartment. GILT facilitates MHC class II–restricted antigen processing by catalyzing the reduction of disulfide bonds of the proteins and thereby exposing hidden epitopes for MHC class II binding (171). Also unknown are the precise roles of PDI, tapasin, and ERp57 in optimal peptide loading. Conceivably, thiol modifications other than disulfide bond formation, such as *S*-glutathionylation and *S*-nitrosylation, are involved in regulation of antigen processing. An understanding of the mechanisms by which thiol-based redox reactions regulate antigen processing is just beginning to emerge. Elucidating the details of redox regulation and the potential roles of other thiol modifications should provide leads for the design of novel therapeutic interventions against intracellular pathogens and tumors.

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Abbreviations

ABC, ATP-binding cassette; BAP31, B-cell receptor–associated protein 31; BH, bleomycin hydrolase; BiP, binding immunoglobulin protein; β_2m, β_2-microglobulin; CTL, cytotoxic T lymphocyte; ER, endoplasmic reticulum; ERAAP, ER aminopeptidase associated with antigen processing; ERAD, ER-associated degradation; Ero1, endoplasmic reticulum oxidoreductin 1; ERdj, endoplasmic reticulum–localized DnaJ homologue; ERp, ER protein; FAD, flavine adenine dinucleotide; GILT, γ-IFN–inducible lysosomal thiol reductase; GrP94, glucose-regulated protein 94; GSH, glutathione; GSSG, glutathione disulfide; HCMV, human cytomegalovirus; HIV, human immunodeficiency virus; HLA, human leukocyte antigen; Hsp, heat-shock protein; Ig, immunoglobulin; LAP, leucine aminopeptidase; LMP, low-molecular-mass polypeptide; LPS, lipopolysaccharide; MECL-1, multicatalytic endopeptidase complex–like-1; MHC, major histocompatibility complex; MMTS, methanethiosulfonate; NEM, *N*-ethylmaleimide; NF-κB, nuclear factor kappa B; PDI, protein disulfide isomerase; PLC, peptide-loading complex; PSA, puromycin-sensitive aminopeptidase; ROS, reactive oxygen species; TAP, transporter associated with antigen processing; TCR, T-cell receptor; TMX, transmembrane Trx-related protein; TPP II, tripeptidyl peptidase II; UGGT, UDP-glucose/glycoprotein glucosyltransferase; UGT, UDP-glucuronosyltransferase; UPR, unfolded protein response.

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