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FORUM REVIEW ARTICLE

Disulfide Reduction in the Endocytic Pathway: Immunological Functions of Gamma-Interferon-Inducible Lysosomal Thiol Reductase

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Abstract

Gamma-interferon-inducible lysosomal thiol reductase (GILT) is constitutively expressed in most antigen presenting cells and is interferon γ inducible in other cell types *via* signal transducer and activator of transcription 1. Normally, N- and C-terminal propeptides are cleaved in the early endosome, and the mature protein resides in late endosomes and lysosomes. Correspondingly, GILT has maximal reductase activity at an acidic pH. Monocyte differentiation *via* Toll-like receptor 4 triggers secretion of a disulfide-linked dimer of the enzymatically active precursor, which may contribute to inflammation. GILT facilitates major histocompatibility complex (MHC) class II-restricted processing through reduction of protein disulfide bonds in the endocytic pathway and is hypothesized to expose buried epitopes for MHC class II binding. GILT can also facilitate the transfer of disulfide-containing antigens into the cytosol, enhancing their cross-presentation by MHC class I. A variety of antigens are strongly influenced by GILT-mediated reduction, including hen egg lysozyme, melanocyte differentiation antigens, and viral envelope glycoproteins. In addition, GILT is conserved among lower eukaryotes and likely has additional functions. For example, GILT expression increases the stability of superoxide dismutase 2 and decreases reactive oxygen species, which correlates with decreased cellular proliferation. It is also a critical host factor for infection with *Listeria monocytogenes. Antioxid. Redox Signal.* 15, 657–668.

Introduction

THE PRIMARY ESTABLISHED ROLE for gamma-interferon-inducible lysosomal thiol reductase (GILT) is to facilitate major histocompatibility complex (MHC) class II-restricted antigen processing, which generates cell surface MHC class II-peptide complexes essential for the activation of $CD4^+$ T lymphocytes [reviewed in (53)]. A brief description of this pathway is presented first. As shown in Figure 1, MHC class II α and β chains are synthesized and form heterodimers in the endoplasmic reticulum, where they associate with invariant chain (Ii). The N-terminal cytoplasmic domain of Ii sorts the class II-Ii complex into the endocytic pathway, where Ii is sequentially cleaved, leaving the class II-associated Ii peptide (CLIP) associated with the class II peptide binding groove. In the acidic environment of the lysosomes, cathepsins are generally activated by autocatalytic cleavage of a pro-peptide that inhibits the activity of the precursor form. Cathepsins are responsible for the proteolysis of endocytosed or phagocytosed exogenous proteins, and endogenous proteins localized to lysosomes or phagosomes, which generates class II binding peptides. The class II-related molecule HLA-DM (H2-M in mice), which is localized in the endocytic pathway, catalyzes the exchange of CLIP for locally generated peptides and stabilizes class II until a high affinity peptide is bound, and thus functions as a peptide editor. Human leukocyte antigen (HLA)-DO (H2-O in mice) associates with HLA-DM in B cells, dendritic cells (DCs), and thymic epithelial cells, and downmodulates HLA-DM function. MHC class II-peptide complexes are directed to the cell surface where they can serve to activate $CD4^+$ T cells. In the absence of inflammation, MHC class II-restricted processing is restricted to professional antigen presenting cells (APCs). Interferon (IFN)-γ induces MHC class II expression on additional cell types such as endothelial cells and some tumors, including melanoma.

Reduction of disulfide bonds in antigens is an important step in MHC class II-restricted processing and presentation. Destabilizing protein structure by acidification and disulfide bond reduction can allow MHC class II binding to the full-length protein or a protein fragment (27, 56). In addition, multiple epitopes require disulfide bond reduction for efficient stimulation of T cells (11, 26, 28). Reduction facilitates lysosomal proteolytic digestion of antigens and generation of antigenic peptides bound to MHC class II for T

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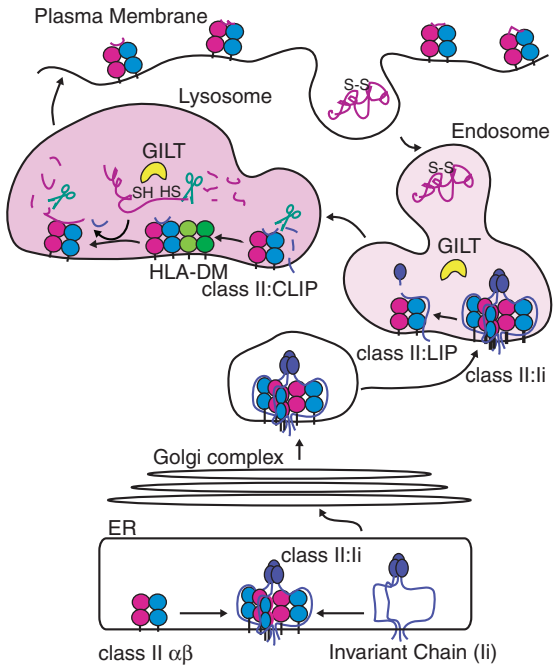


FIG. 1. GILT in the MHC class II processing pathway. In the ER, MHC class II α (red) and β (light blue) chains are synthesized, form heterodimers, and associate with Ii (dark blue). The cytoplasmic tail of Ii directs the class II-Ii complex into the endocytic pathway. Cathepsins (represented by scissors) are responsible for the proteolysis of protein antigens and the sequential cleavage of Ii leaving CLIP bound in the class II peptide binding groove. Mature GILT (yellow) is localized to the late endosomes and lysosomes where it catalyzes reduction of protein disulfide bonds. HLA-DM (green) catalyzes the exchange of CLIP for locally generated peptides and functions as a peptide editor. MHC class II-peptide complexes are directed to the cell surface where they can stimulate $CD4^+$ T cells. CLIP, class II-associated invariant chain peptide; ER, endoplasmic reticulum; GILT, gamma-interferon-inducible lysosomal thiol reductase; Ii, invariant chain. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

cell stimulation (11). Intracellular reducing activity is primarily associated with lysosomes (11). However, disulfide bond reduction is not chemically favored at the acidic pH found in the lysosomal compartment, and it has been suggested that lysosomes may be oxidizing rather than reducing (4). GILT is the only known reductase localized to the endocytic pathway and catalyzes disulfide bond reduction in this compartment. Lysosomal proteases have the ability to both generate and destroy antigenic epitopes (67), and MHC class II binding can protect the bound epitope from proteolysis (40, 61). We propose that GILT facilitates MHC class II-restricted antigen processing by reducing substrates in this compartment and exposing constrained epitopes for MHC class II binding, thus protecting them from protease digestion. Here, we review GILT's contributions to the immune response, focusing mainly on its role in MHC class II-restricted processing.

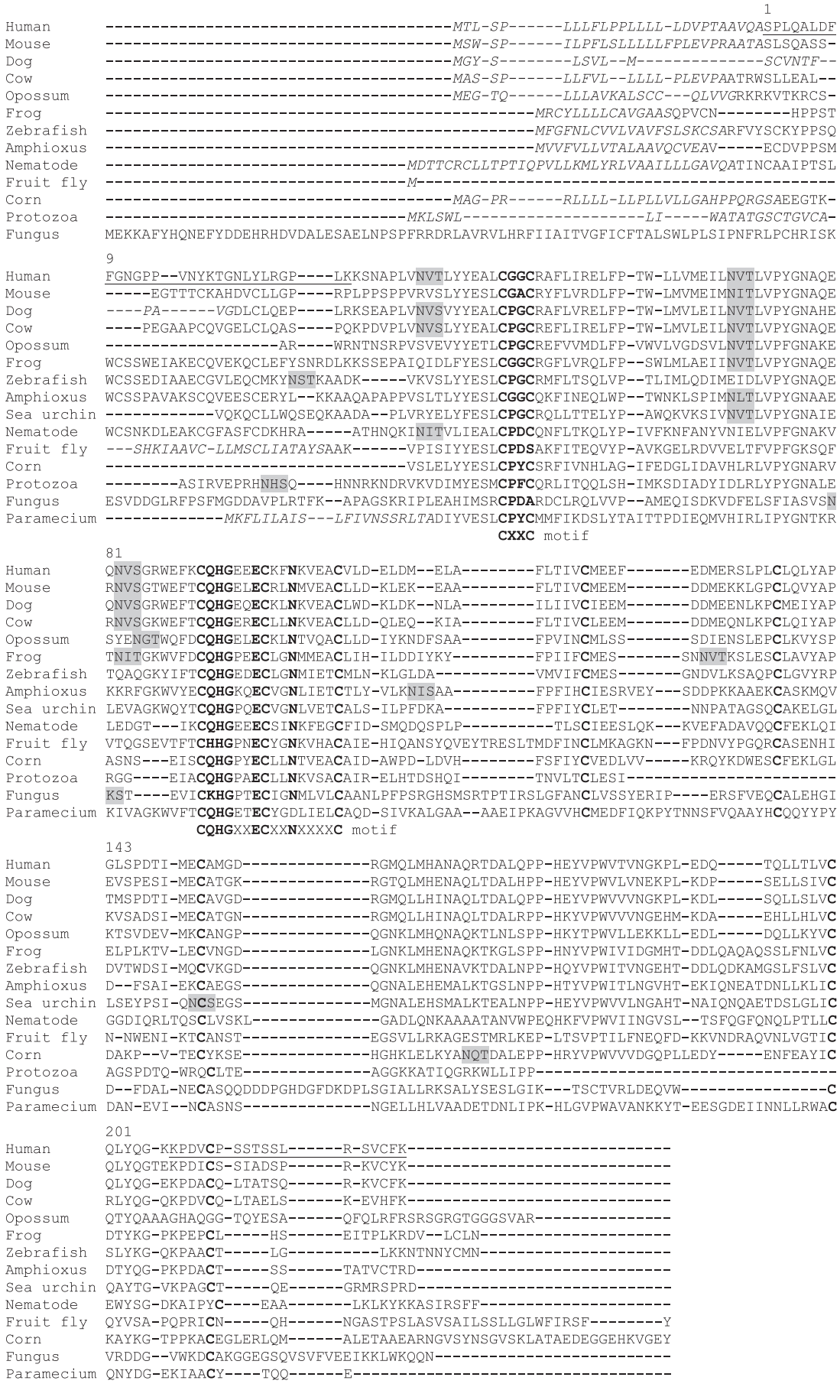
GILT Expression

Luster *et al.* initially described GILT as an IFN-γ-inducible protein (IP-30) (34). GILT is constitutively expressed in most APCs, including monocytes/macrophages, B cells (primary and cell lines), and bone-marrow derived DCs (3, 25, 29, 30, 34, 36). GILT is also constitutively expressed in thymocytes (37), mature T cells (5, 37), and some fibroblasts (9, 64). IFN-γ plays an important role in inducing expression of MHC class II and other components of the class II-restricted processing pathway, including Ii and HLA-DM (10). IFN-γ rapidly induces GILT in many cell types, including immature monocytes and monocyte precursors, other fibroblasts, human umbilical vein endothelial cells, and melanoma cell lines, with mRNA detectable within 30 min to 2 h and maximal protein expression at 48 h (21, 29, 30, 34). IFN-γ signals through a specific cell surface receptor followed by activation of Janus kinases 1 and 2 and signal transducer and activator of transcription (STAT) 1. IFN-γ-inducible class II expression is controlled by IFN-γ-inducible isoforms of class II transactivator, although neither basal nor IFN-γ-inducible GILT expression is mediated by class II transactivator (41). However, STAT1 is required for IFN-γ-inducible GILT expression in melanoma and fibrosarcoma cell lines, and STAT1 binds oligonucleotides from predicted STAT1 binding regions in the GILT promoter (41). Consistent with this mechanism, IFN-γ-induced GILT mRNA expression in hematopoietic cells does not require *de novo* protein synthesis (29, 34). Additionally, macrophage differentiation has been identified as a distinct pathway of induction of GILT expression. Treatment with phorbol-12-myristate-13-acetate or Toll-like receptor (TLR) 4 ligands such as lipopolysaccharide or whole *E. coli* bacteria induces differentiation of immature monocytes and monocyte precursors as demonstrated by adherence, secretion of pro-inflammatory cytokines and upregulation of antigen processing and presentation components, including GILT (29, 30, 34). TLR4-mediated induction of GILT protein continues to increase at 60 h, which is delayed in comparison to IFN-γ-induced expression (29). In contrast to induction by IFN-γ, TLR4-induced GILT mRNA expression requires *de novo* protein synthesis, nuclear factor kappa B signaling, and secretion of inflammatory cytokines (interleukin-1β and tumour necrosis factor) (29).

Biochemical Characterization of GILT

Human GILT is composed of 261 amino acids with a 37 amino acid signal sequence and a 224 amino acid precursor form (Fig. 2). The 35 kDa precursor is tagged with mannose-6-phosphate (M6P) residues and targeted to the endocytic

FIG. 2. Alignment of GILT homologs. GILT protein homologs from multiple representative organisms were aligned using NCBI Cobalt. The signal sequences predicted by SignalP 3.0 are shown in italics. N-linked glycosylation sites predicted using NetNGlyc 1.0 are shaded. The N- and C-terminal pro-peptides determined in human GILT are underlined. The 10 cysteine residues that are conserved in the majority of species; the reductase active site and the GILT motif are shown in bold. Residue numbers correspond to human GILT. Cys-46 and Cys-49 are part of the CXXC reductase active site. Cys-91 through Cys-106 defines the GILT motif $CQHGX_2ECX_2NX_4C$.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Human
Mouse
Dog
Cow
Opossum
Frog
Zebrafish
Amphioxus
Nematode
Fruit fly
Corn
Protozoa
Fungus
Paramecium
9
81
CXXC motif
143
CQHGXXECXXNXXXXC motif
201

pathway *via* the M6P receptor (M6PR) (3, 36). In early endosomes, N- and C-terminal pro-peptides are cleaved to generate a 28 kDa mature form (Fig. 1) (44). *In vitro*, the C-terminus can be cleaved by cathepsin (Cat) B and CatL and partially cleaved by CatS, and the N-terminus is cleaved by CatS, CatL, and CatD (44). However, CatB and CatS are dispensible *in vivo*, as the kinetics of GILT maturation is not significantly delayed in $CatB^{-/-}$ or $CatS^{-/-}$ B cells (25). CatS does play a role in degradation of mature GILT (25). The mature form of GILT is localized to late endosomes and lysosomes and has maximal reductase activity at the acidic pH found in these compartments (3, 36). A thioredoxin-like CXXC motif, corresponding to Cys-46 and Cys-49 in human GILT, constitutes the reductase active site (Fig. 2) (3). Similar to reduction by thioredoxin, the N-terminal Cys-46 thiol group initiates a nucleophilic attack on a disulfide bond (Fig. 3) (44). This results in the formation of a GILT-substrate mixed disulfide intermediate, with a subsequent intramolecular attack by the Cys-49 thiol, resulting in the release of the reduced substrate (Fig. 3) and oxidized GILT, which must be reduced before catalyzing the next reaction (44). Any specific characteristics that delineate substrate specificity remain to be determined.

A portion of precursor GILT is secreted as an enzymatically active disulfide-linked dimer (29, 30, 44). In B cells constitutively synthesizing GILT or in IFN-γ-treated monocytes, intracellular mature GILT is the dominant form (3, 29). However, in TLR4-stimulated monocytes the majority of GILT generated is secreted as the precursor rather than being transported to and maturing in the lysosomes (29, 30). Indeed, GILT levels are increased in the serum of mice following sublethal LPS injection (30). This distinct pattern of GILT induction is due to regulation of the enzymes involved in M6P tagging, evidenced by reduced transcription of both the γ subunit of *N*-acetylglucosamine-1-phosphotransferase and particularly the uncovering enzyme (30) (Fig. 4). This results in a large pool of GILT that lacks M6P and localization of GILT in peripheral vesicular structures consistent with constitutive secretory vesicles (30). It may represent a generalized mech-

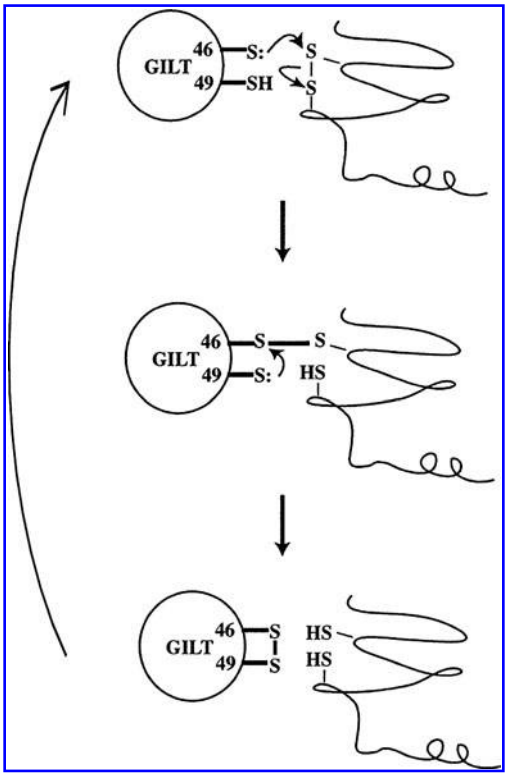

FIG. 3. Proposed mechanism of GILT-mediated reduction. Similar to thioredoxin, the thiol group of the N-terminal cysteine (Cys-46 in human GILT) initiates a nucleophilic attack on the substrate disulfide bond. There is formation of a mixed disulfide GILT-substrate intermediate, which can be isolated using a trapping mutant in which the C-terminal cysteine has been mutated. Subsequent intramolecular attack by the Cys-49 thiol results in the release of the reduced substrate. Lysosomal cysteine is a physiological reducing agent that is capable of reducing GILT, so that it can catalyze the next reaction. Adapted from (44).

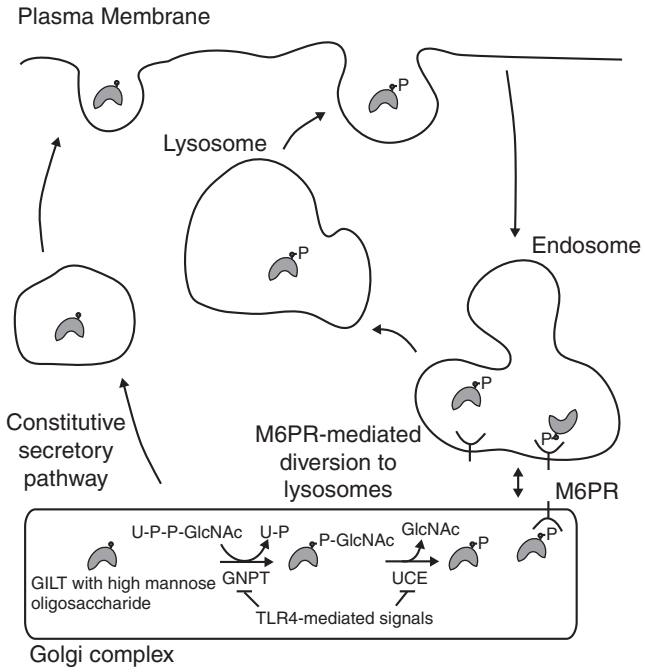

FIG. 4. GILT trafficking and secretion. Lysosomal enzymes are synthesized in the ER and transported through the Golgi complex to the *trans*-Golgi network. From the *trans*-Golgi network, proteins such as GILT can follow the constitutive secretory pathway (*left*) to the plasma membrane with possible endocytosis to reach the lysosome, or they can directly traffic to the lysosome *via* the M6PR (*right*) [reviewed in (48, 55)]. The specific *N*-glycans that are mannose 6-phosphorylated to mediate the lysosomal trafficking of GILT have not been characterized. In the ER and early Golgi compartments, GNPT catalyzes the transfer of GlcNAc-1-phosphate from UDP-GlcNAc to certain C6 hydroxyl groups of mannose sugars on the α-1,6 branch. A subsequent phosphorylation may occur on the α-1,3 branch. In the TGN, UCE hydrolyzes the phosphodiester bond releasing GlcNAc and exposing the M6P, which is recognized by M6PRs. M6PRs mediate transport of M6P-tagged lysosomal enzymes to endosomes. M6PRs dissociate from their ligand in the mildly acidic environment of the early endosomes and return to the TGN to mediate additional rounds of transport. Following Toll-like receptor-mediated signals *via* nuclear factor kappa B, reduced transcription of GNPT and UCE reduces M6P tagging and results in a shift toward the constitutive secretory pathway. GNPT, *N*-acetylglucosamine-1-phosphotransferase; M6PR, mannose-6-phosphate receptor; UCE, uncovering enzyme.

anism used by differentiated macrophages for the secretion of soluble lysosomal enzymes that mediate extracellular degradation. The findings also suggest a possible role for GILT in TLR4-mediated inflammatory responses. *In vitro* reductase activity has been measured using ^{125}I-F(ab′)$_2$ or Bodipy-FL-cystine as a substrate, and intracellular reductase activity has been demonstrated using internalized ^{125}I-labelled anti-CD63 mAb (3, 30). Dithiothreitol, cysteine, and cysteinyl glycine, but not glutathione, are capable of regenerating active precursor and mature GILT *in vitro* (3, 44). Cysteine may be responsible for regenerating GILT *in vivo*, as a specific transport system to transfer cysteine into the endosomes and lysosomes of APCs has been described (16, 47).

As illustrated in Figure 2, human GILT has 11 conserved cysteine residues. Mutational analyses have demonstrated that Cys-222 is responsible for disulfide-mediated dimerization of secreted precursor GILT (45). Mutations of Cys-91, -98, -200, or -211 impair processing to the mature form (23, 45). Remarkably, although Cys-211 is present in the C-terminal pro-peptide and presumably nonessential to the function of the mature form, C211S GILT and a mutant in its proposed disulfide partner, C200S GILT, are impaired in processing to the mature form, although the mutant precursors remain active especially at neutral pH (45). Cysteine to serine mutation of Cys-106, Cys-122, Cys-136, and Cys-152 results in mutants that are not expressed, and these residues are presumably involved in internal disulfide bonds required for proper folding (45). The active site cysteines are also involved in GILT maturation, as mutation of Cys-46 or Cys-49 diminishes processing of the precursor to the mature form (23). GILT's reductase active site may autocatalyze reduction of the precursor form to expose the dibasic cleavage sites flanking the pro-peptide sequences to lysosomal cathepsins. Alternatively, GILT's reductase active site may play an indirect role in maturation by altering the expression of, or maintaining the activity of, lysosomal proteases that are responsible for cleavage of GILT's N- and C-terminal pro-peptides (19).

Role in MHC Class II-Restricted Antigen Presentation

The most well-described function of GILT is enhancing MHC class II-restricted antigen processing. Maric *et al.* generated GILT$^{-/-}$ mice, which have defects in MHC class II-restricted antigen processing (36), even though GILT-deficient APCs have normal expression of MHC class II (23, 36). Hen egg lysozyme (HEL) is an excellent model antigen for evaluating the role of protein structure in antigen processing, because it has four intrachain disulfide bonds (22), it is resistant to proteolytic cleavage without prior reduction (66), and multiple I-A^b-restricted epitopes have been described (59) (Fig. 5). Intracellular processing of HEL to generate the I-A^b-restricted HEL peptide involving residues 74–88 (HEL$_{74–88}$) is GILT-dependent in splenocytes, B cells, and DCs (23, 36) (and unpublished data). Prior reduction of HEL with purified GILT reconstitutes the ability of GILT-deficient APCs to present this epitope (36). HEL$_{74–88}$ contains two cysteines that are each involved in a disulfide bond (22) (Fig. 5), and earlier studies support the need for disulfide bond reduction for presentation of this epitope (28). Processing of the HEL epitope composed of residues 46–61, which do not contain any disulfide bonds, was partially diminished in GILT$^{-/-}$ APCs (36). This effect may be due to the fact that residues 74–88 contact residues 46–61 in the native HEL structure and may make residues 46–61 less accessible (Fig. 5). In contrast, the processing of two I-A^b-restricted HEL epitopes involving residues 20–35 and 30–53, which share one cysteine involved in a disulfide bond, is not affected by the absence of GILT (23, 36), perhaps because the topology of these epitopes renders them accessible to class II binding without reduction by GILT or acidic pH alone is sufficient to denature this region for class II binding (Fig. 5). Therefore, a disulfide-bond-containing antigen may have some epitopes that are GILT dependent and others that are not. GILT dependence is not specifically correlated with cysteines involved in disulfide bonds being present in the epitope, but rather thought to depend on whether the epitope requires reduction to be exposed for class II binding. Despite the fact that not all HEL epitopes require GILT, the overall CD4$^+$ T cell response to HEL in GILT$^{-/-}$ mice is reduced by 90% compared to that in wild-type mice (36). Similar reductions in recall responses are seen upon immunization with other proteins containing disulfide bonds, such as bovine ribonuclease A and human immunoglobulin G (36). Only a slight difference is observed after immunization with bovine α-casein, an antigen that does not contain disulfide bonds (36).

The reductase activity of GILT is essential for its function in MHC class II-restricted processing. Mutation of either Cys-46 or Cys-49 of the CXXC reductase active site, either individually or together, eliminates efficient intracellular processing of the GILT-dependent HEL$_{74–88}$ epitope (23). Consistent with the proposed mechanism (Fig. 3), no intracellular processing

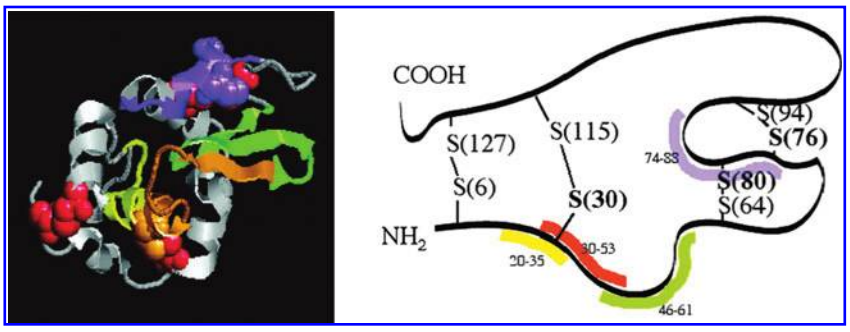

FIG. 5. Hen egg lysozyme structure and position of I-A^b restricted epitopes. Ribbon diagram (*left*) and schematic (*right*) of X-ray crystal structure of hen egg lysozyme demonstrating the location of disulfide bonds and I-A^b-restricted epitopes: residues 20–35 in yellow, 30–53 in orange, 46–61 in green, and 74–88 in purple. Cysteine residues, all of which are involved in disulfide bonds, are shown with balls in red, unless they are part of an epitope as described above. Reprinted with permission from Maric *et al.* (36). (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

is detected in the absence of the N-terminal active site Cys-46, which initiates nucleophilic attack on the substrate disulfide bond (23). T cell responses are reduced ~10-fold with C49S GILT (23), in which case Cys-46 can still generate a mixed disulfide intermediate and a low level of reduction and substrate release may be mediated by an alternate reducing agent such as lysosomal cysteine.

Melanocyte differentiation antigens, including tyrosinase, tyrosinase-related protein (TRP) 1, TRP2, and gp100, represent a clinically important group of antigens expressed by benign melanocytes and malignant melanoma. These integral membrane proteins involved in melanin pigment synthesis are targets of the immune response in melanoma and autoimmune skin depigmentation (vitiligo). Melanoma (www.cancerimmunity.org/peptidedatabase/differentiation.htm) and vitiligo (31, 35, 42, 43) patients generate T cells specific for these antigens. Since these antigens are presented on MHC class II and contain disulfide bonds (7, 17, 39), they are likely to require GILT for efficient class II-restricted processing. In fact, the class II-restricted processing of an epitope from human tyrosinase involving residues 56–70 is partially GILT dependent (21, 32), and an epitope from murine TRP1 residues 109–130 is strongly GILT dependent *in vitro* (49). Further, GILT in APCs accelerates TRP1-specific $CD4^+$ T cell-mediated vitiligo *in vivo* (49). The appearance of vitiligo correlates with increased TRP1-specific T cells with an effector memory phenotype and TRP1-specific effector memory T cells are increased in the presence of GILT (49), suggesting that efficient class II-restricted processing of TRP1 in the presence of GILT enhances T cell activation and the development of vitiligo.

Melanoma is one of the tumors that may aberrantly express MHC class II molecules. As class II-expressing melanoma cells can present epitopes from melanoma-associated antigens (51, 52, 65, 68), they can potentially stimulate anti-melanoma $CD4^+$ T cell responses. However, class II expression in melanoma correlates with advanced disease and poor survival (54, 71). The mechanism of this apparent paradox is unclear. Components of the class I pathway are targets of immune evasion used by melanoma (6, 15, 50, 58, 69) and viral pathogens [reviewed in (20)]. One explanation could be a defect in the expression of a component of class II processing, such as GILT. Consistent with this hypothesis, a study of 10 class II-expressing human melanoma cell lines revealed little or no GILT expression (21). The expression of class II by melanoma cells in the absence of GILT may be a mechanism of immune evasion, as such cells may be unable to process the GILT-dependent epitopes presented by professional APCs, which activate the T cell repertoire. Currently, there is no *in vivo* evidence to support the loss of functional GILT in melanoma. In addition, we are not aware of any pathogens that block GILT function.

Additional class II epitopes have been evaluated for the requirement of GILT-mediated reduction. Class II-restricted presentation of a cysteinylated peptide derived from human IgG κ residues 188–203, in which Cys-194 forms an intrachain disulfide bond, is GILT dependent (21, 32). Another example comes from the human immunodeficiency virus-1 envelope protein. Two epitopes straddle two different cysteine residues at the base of the V1/V2 loops connecting two antiparallel β sheets. Recognition of one of these epitopes is partially GILT dependent, whereas recognition by the other is similar in the absence of GILT (57). The influenza hemagglutinin major subunit, which has four intrachain disulfide bonds and is connected to the virion with one interchain disulfide bond, has also been investigated for GILT-dependence. Although the site 1 hemagglutinin epitope involving residues 107–119 requires disulfide bond reduction for presentation on class II, it does not require GILT (64), suggesting a GILT-independent mechanism of endosomal reduction.

A recent study used mass spectrometry to evaluate the impact of GILT on the overall repertoire of MHC class II-bound peptides eluted from wild-type and $GILT^{-/-}$ resting splenocytes (8). Surprisingly, no unique peptides were identified from GILT-containing APCs, and only 5.5% of peptides are more abundant in wild-type APCs. In contrast, 94.5% of peptides are more abundant on $GILT^{-/-}$ APCs including 2% which are exclusively identified on $GILT^{-/-}$ APCs and 3.5% which are 10- to 60-fold more abundant in $GILT^{-/-}$ APCs. Despite the fact that GILT is required for the efficient presentation of some antigens, the range of class II peptides is not dramatically altered at steady state. In fact, GILT may diminish the steady state presentation of self-antigens. Consistent with the notion that GILT exposes buried regions of the antigens' tertiary structure

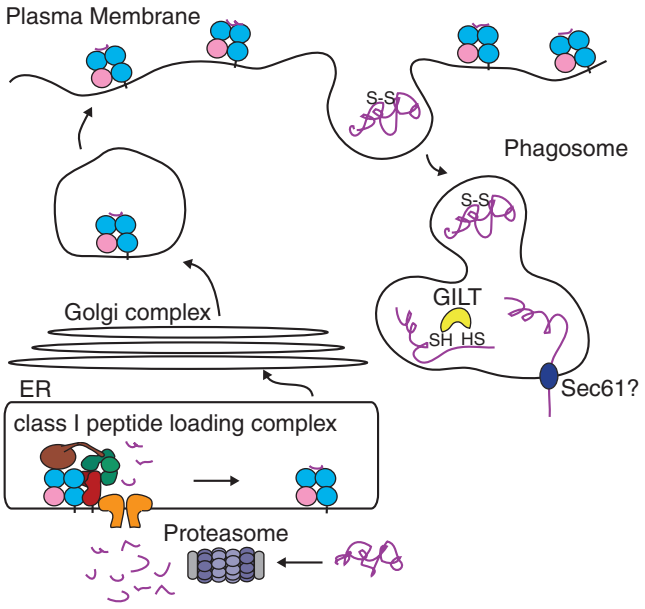

FIG. 6. The role of GILT in the antigen cross-presentation pathway. The cytosolic pathway of cross-presentation in dendritic cells involves the transfer of exogenous proteins or protein fragments (purple) from the phagosome into the cytosol [reviewed in (2)]. Large proteins in the phagosome must be unfolded and/or partially cleaved before retrotranslocation into the cytosol, possibly *via* Sec61 or Derlin-1 recruited from the ER. For disulfide bond containing antigens, reduction by GILT facilitates the unfolding, proteolysis, and retrotranslocation steps. Once in the cytosol, these proteins are degraded by the proteosome, and the resulting peptides are translocated into the ER *via* the TAP transporter (orange). Peptides of the appropriate sequence and length bind MHC class I molecules in the peptide loading complex composed of class I heavy chain (light blue), β_2m (pink), tapasin (red), ERp57 (green), and calreticulin (brown). Peptide binding triggers dissociation of the peptide loading complex, and the class I:peptide complexes are directed to the plasma membrane. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

for class II binding, most of the class II bound peptides identified in the GILT$^{-/-}$ sample are derived from the proteins' extreme N- or C-termini. It is likely that these regions have less structural constraints and are preferentially accessible for class II binding and proteolytic cleavage.

Role of GILT in MHC Class I-Restricted Cross-Presentation

A more recently described immunological function for GILT is the enhancement of cross-presentation of MHC class I-restricted epitopes derived from viral glycoproteins (62). Cross-presentation refers to the processing of exogenous antigens for presentation by MHC class I molecules to CD8$^+$ T cells. Priming naïve CD8$^+$ T cells to infectious agents such as viruses depends on the ability of host APCs, primarily DCs, to phagocytose virally infected cells and generate complexes of peptides derived from viral antigens with MHC class I molecules (60). The major mechanism of cross-presentation involves the transfer of the antigens or fragments of the antigens from the DC phagosome into the cytosol (1) (Fig. 6). There the antigens are degraded by the proteasome, the resulting peptides are translocated into the endoplasmic reticulum *via* the TAP transporter and those with the appropriate sequence and length bind to newly synthesized MHC class I molecules.

Large protein antigens in the phagosome must be unfolded and/or partially proteolysed for translocation into the cytosol (18), and when the antigens contain disulfide bonds their reduction may therefore be required. For glycoprotein B (gB), a major envelope glycoprotein of herpes simplex virus 1 that encodes the dominant MHC class I-restricted epitope of the virus in H2^b mice (Fig. 7), cross-presentation is in fact GILT-dependent (62). Examination of a space filling model of gB demonstrates that this GILT-dependent epitope is buried within the three-dimensional structure (Fig. 7). DCs from GILT$^{-/-}$ mice are unable to generate the epitope *in vitro,* and GILT$^{-/-}$ mice develop a reduced CD8$^+$ T cell response to the epitope when they are infected with herpes simplex virus 1. Similar to the findings with HEL and the class II response, the ability of GILT$^{-/-}$ DCs to cross-present the gB epitope is restored by expression of wild-type GILT but not by GILT with mutated active-site cysteines. In these experiments phagosomal proteolysis is also required for cross-presentation, suggesting that GILT-mediated reduction may facilitate proteolysis in the phagosome. Additional *in vivo* infection experiments suggest that the development of CD8$^+$ T cell responses to influenza virus glycoproteins (hemagglutinin and neuraminidase) also exhibit a degree of GILT dependence.

Summary of the Roles of GILT in Antigen Processing

GILT is critical for the processing of disulfide-containing antigens and the presentation of a subset of peptides derived from them to T cells. It is therefore important in the development of the overall T cell response to protein antigens that contain disulfide bonds. The constitutive expression of GILT

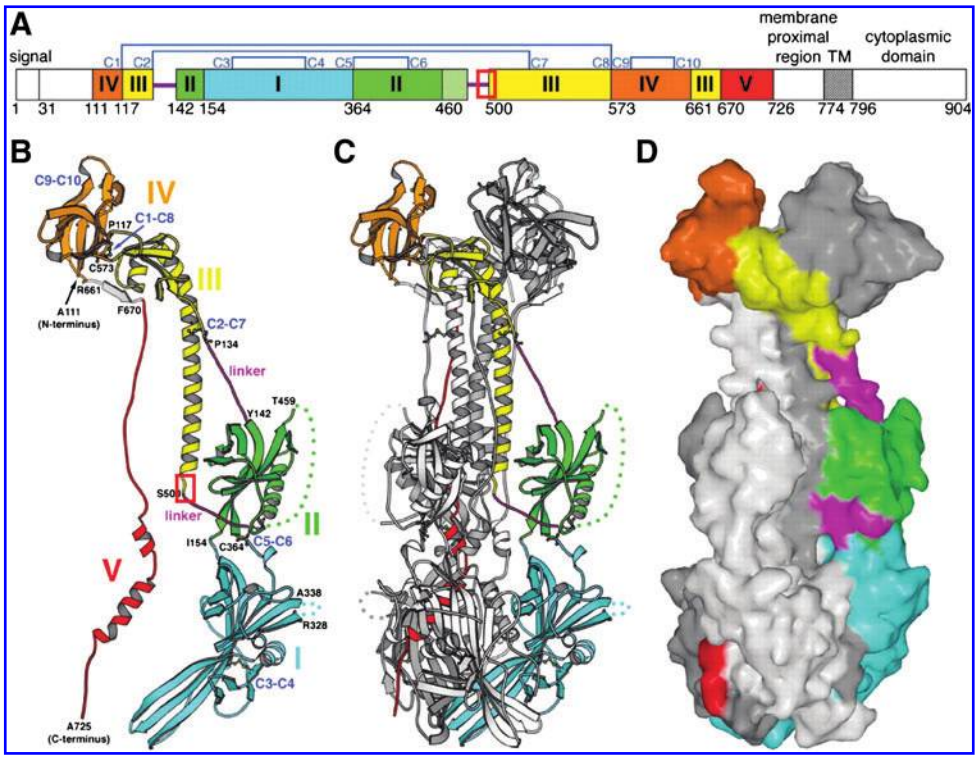

FIG. 7. gB from herpes simplex virus 1. gB is a trimeric glycoprotein that contains a peptide (residues 498–505) that binds to the murine MHC class I molecule H2-K^b and is recognized by CD8$^+$ T cells. The location of the peptide is indicated by a red box in **(A)**, representing the protein in linear form, and in the structural depiction of a single gB subunit in **(B)**. Generation of this peptide in association with H2-K^b by the pathway shown in Figure 6 is dependent on the presence of GILT in the phagosome. **(C)** and **(D)** show the structure of the gB trimer as a ribbon diagram and a space filling model, respectively, and indicate that the H2-K^b-binding peptide is buried in the three-dimensional structure of the gB molecule. Reprinted with permission from Heldwein *et al.* (24). gB, glycoprotein B. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

in APCs is likely to account for enhanced reduction of proteins in the late endosomal, lysosomal, and phagosomal compartments. A combination of GILT-mediated reduction and proteolysis in the these compartments can enhance the generation of class II epitopes as well as facilitate the translocation of internalized antigens into the cytosol for proteosomal processing and cross-presentation.

Phylogeny

The *IFI30* gene encoding GILT is conserved among eukaryotes (46). GILT homologs have been identified in more than 45 eukaryotes (Table 1). Each GILT protein sequence from primates through paramecium contains six conserved cysteines and a signature sequence $CQHGX_2ECX_2NX_4C$ of unknown function (Fig. 2). Most GILT homologs have 10 conserved cysteines including the CXXC reductase active site (Fig. 2). The C-terminal cysteine of the reductase active site is not conserved in insects or fungus (Fig. 2), which also suggests that GILT may have functions other than reduction. The appearance of GILT homologs in the most primitive eukaryotes, and long before the development of adaptive immunity in jawed fish, suggests that GILT has a fundamental role in cellular processes and was adapted to facilitate antigen processing.

Table 1. Gamma-Interferon-Inducible Lysosomal Thiol Reductase Homologs

Species	*Common name*	*NCBI accession number*	*Reference*
**Homo sapiens*	Human	NP_006323	34
Pan troglodytes	Chimpanzee	XP_001162304	
Macaca mulatta	Rhesus monkey	XP_001114116	
Callithrix jacchus	White-tufted-ear Marmoset	XP_002761946	
**Mus musculus*	Mouse	NP_075552	36
Rattus norvegicus	Rat	NP_001025197	
**Canis familiaris*	Dog	XP_533874	
**Bos taurus*	Cow	NP_001094721	
Sus scrofa	Pig	NP_001124518	13
Equus caballus	Horse	XP_001500606	
Gallus gallus	Chicken	XP_418246	
**Monodelphis domestica*	Opossum	XP_001368439	
**Xenopus tropicalis*	Frog	NP_001017196	
**Danio rerio*	Zebrafish	NP_001006057	70
Epinephelus coioides	Orange-spotted grouper	ABS19625	12
Tetraodon nigroviridis	Puffer fish	CR697192	
Larimichthys crocea	Large yellow croaker	ABB87180	73
Anoplopoma fimbria	Sable fish	ACQ58973, ACQ58865	
Salmo salar	Atlantic salmon	ACI69367	
Ictalurus punctatus	Catfish	ABC75582	
Sparus aurata	Gilthead seabream	AM920662	38
**Strongylocentrotus purpuratus*	Sea urchin	XP_791549	
Haliotis discus discus	Disk abalone	ABQ24037	14
Penaeus monodon	Prawn	ACJ23247	
Pinctada fucata	Pearl oyster	ACX30641	72
**Branchiostoma belcheri tsingtauense*	Amphioxus	AAQ83892	33
Ciona intestinalis	Vase tunicate	XP_002120789	
**Caenorhabditis elegans*	Nematode	NP_496397	
Brugia malayi	Filariasis nematode	XP_001902931	
Aedes aegytpi	Yellow fever mosquito	ABF18298	
Anopheles gambiae	Malaria mosquito	XP_313849	
**Drosophila melanogaster*	Fruit fly	NP_650287	
Lepeophtheirus salmonis	Salmon louse	ACO12961, ADD38067	
Ixodes scapularis	Tick	XP_002434860, XP_002412281	
Gloassina morsitans morsitans	Tsetse fly	AAD20155	
Pediculus humanus corporis	Human body louse	XP_002433162, XP_002428434	
Bombyx mori	Silkworm	NP_001103767	
Apis mellifera	Honey bee	XP_001121957	
**Arabidopsis thliana*	Thale cress	NP_563779, NP_193023, NP_567395, NP_193032, NP_193026, NP_001154228	
Zea mays	Corn	NP_001151695	
Oryza sativa	Rice	ABF95436	
Ricinus communis	Castor oil plant	XP_002528051, XP_002526664, XP_002526663	
Phaeodactylum tricornutum	Diatom	XP_002177956	
**Toxoplasma gondii*	Protozoa	EEE32163	
**Aspergillus clavatus*	Fungus	XP_001275855	
**Paramecium tetraurelia*		XP_001347184	
Perkinsus marinus	Oyster parasite	XP_002788782, XP_002785541	

*Indicates inclusion in protein alignment in Figure 2.

Cellular Redox State and Infection

In addition to GILT's defined role in antigen processing, GILT has been found to regulate the cellular redox state. GILT increases the expression and stability of superoxide dismutase 2, a mitochondrial enzyme responsible for the conversion of superoxide radical into hydrogen peroxide (9). GILT-expressing fibroblasts have decreased levels of reactive oxygen species such as superoxide anion, and these changes correlate with decreased cellular proliferation (9). Similarly, GILT decreases proliferation and cytotoxic activity in T cells (5). GILT expression levels increase with T cell development from double-positive to single-positive thymocytes to peripheral T cells (37), and this may serve as a mechanism to regulate T cell sensitivity to self-antigens. In fact, $GILT^{-/-}$ mice develop earlier and more severe hyperglycemia in streptozotocin-induced diabetes, a $CD8^{+}$ T cell-mediated model of autoimmunity, which shows that GILT serves to diminish autoimmunity independent of its role in MHC class II-restricted processing (37). The precise molecular mechanism by which GILT expression increases mitochondrial-based superoxide dismutase 2 remains to be determined. GILT may regulate the cellular redox status by regulating cysteine/cystine balance.

GILT is also a critical host factor that facilitates the activity of bacterial hemolysins. During infection with the intracellular bacterium *Listeria monocytogenes,* the bacterium is phagocytosed by macrophages and evades destruction by using its pore-forming hemolysin listeriolysin O (LLO) to escape into the cytoplasm. Reduction is required for LLO activity *in vitro,* and GILT serves this role *in vivo* (63). Bacterial replication in $GILT^{-/-}$ macrophages is diminished due to delayed phagosomal escape (63). A GILT-LLO conjugate can be isolated using a trapping mutant, a cysteine to serine point mutation of the C-terminal cysteine of the CXXC active site (44, 63). This demonstrates that LLO is a substrate for GILT and that GILT reduces LLO using the CXXC active site. Thus, $GILT^{-/-}$ mice are resistant to *L. monocytogenes* infection (63). This function is not limited to LLO. GILT can also activate streptolysin O, a virulence factor of *Streptococcus pyogenes* with pore forming and cytolytic activity (63). Phagocytosis is not essential for activation of hemolysin family members, as secreted precursor GILT is capable of activating streptolysin O (63). GILT may therefore enhance hemolysin-mediated tissue damage. The story of GILT is just beginning. Future studies will undoubtedly discover novel functions for this unusual thiol reductase.

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Abbreviations Used

APC = antigen presenting cell
CLIP = class II-associated invariant chain peptide
DC = dendritic cell
ER = endoplasmic reticulum
gB = glycoprotein B
GILT = gamma-interferon-inducible lysosomal thiol reductase
GNPT = *N*-acetylglucosamine-1-phosphotransferase
HEL = hen egg lysozyme
HLA = human leukocyte antigen
IFN = interferon
Ii = invariant chain
LLO = listeriolysin O
M6P = mannose-6-phosphate
M6PR = mannose-6-phosphate receptor
MHC = major histocompatibility complex
STAT = signal transducer and activator of transcription
TLR = Toll-like receptor
TRP = tyrosinase-related protein
UCE = uncovering enzyme

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