Inhibition of Invariant Chain (Ii)–Calnexin Interaction Results in Enhanced Degradation of Ii but Does Not Prevent the Assembly of αβIi Complexes

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Summary

Calnexin is a resident protein of the endoplasmic reticulum (ER) that associates with nascent protein chains. Among the newly synthesized integral membrane proteins known to bind to calnexin is invariant chain (Ii), and Ii release from calnexin coincides with proper assembly with major histocompatibility complex (MHC) class II heterodimers. Although calnexin association with several membrane glycoproteins depends on interactions involving N-linked glycans, we previously reported that a truncation mutant of mouse Ii (mlil-107) lacking both N-glycosylation sites was highly effective in associating with MHC class II heterodimers and escorting these dimers through the secretory pathway. This could indicate that calnexin, despite binding to both Ii and class II, is not necessary for the proper interaction of these proteins, or that in contrast to most membrane glycoproteins, the N-linked glycans of Ii are not critical to its interaction with this chaperone. To examine this issue, we have directly explored the binding of calnexin to both Ii truncation mutants lacking the typical sites of N-glycosylation or Ii produced in cells treated with tunicamycin to prevent glycan addition. These experiments revealed that either method of eliminating N-linked carbohydrates on Ii also inhibited association with calnexin. A lumenally truncated form of Ii (mlil-131) that still has N-linked carbohydrates showed a decreased affinity for calnexin compared with intact Ii, however, indicating that calnexin–Ii binding is not determined solely by the sugar moieties. All forms of Ii lacking N-linked sugars and showing defective association with calnexin also had enhanced rates of preendosomal degradation. Despite this effect on degradation rate, tunicamycin treatment did not inhibit the association of class II with glycan-free Ii. These data support the view that calnexin is not an absolute requirement for the proper assembly of class II–Ii nonamers, but rather acts primarily to retain Ii in the ER and to inhibit its degradation. These two properties of calnexin–Ii interaction may help ensure that sufficient intact Ii is available for efficient inactivation of the binding sites of newly synthesized class II molecules, while limiting the ability of excess free Ii to alter the transport properties of the early endocytic pathway.

Invariant chain (Ii) is a type II integral membrane glycoprotein that associates with newly synthesized MHC class II chains shortly after their import into the endoplasmic reticulum (ER). Ii forms noncovalent homotrimers (1), each chain of which associates with a class II αβ heterodimer (2). This nonameric structure is competent for movement out of the ER and through the Golgi complex and trans-Golgi network. By either a direct route (3–5) or through a pathway involving the plasma membrane and early endosomes (5–9), the complex moves to more acidic and proteolytic endocytic organelles where Ii is degraded and peptide ligands are acquired by the class II molecules (9–14).

Abbreviations used in this paper: DSP, dithiobis[succinylproprionate]; ER, endoplasmic reticulum; Ii, invariant chain; mlil, mouse Ii.

Several different experimental models have been used to explore the events involved in the initial assembly and intracellular trafficking of class II heterodimers and Ii, as well as the fate of subsets of these chains produced in the absence of one or more of the oligomer subunits. Results obtained from studies in microsome-containing in vitro translation systems (15–17), after transient (18–20) and stable (21) transfection of various fibroblast cell lines and using lymphoid cells from mice whose Ii genes have been disrupted by homologous recombination (22–24), all indicate that class II heterodimers can be formed in the absence of Ii. The extent to which heterodimer assembly occurs in the absence of Ii, however, varies greatly among allelic class II products (20, 25). In addition, cells producing class II α and β chains in the absence of Ii show elevated levels of class II...
proteins in high molecular weight complexes (26), and similar aggregates are seen in normal lymphoid cells at very short times after protein entry into the ER (27). In normal cells with li, these class II chains rapidly leave such aggregates and assemble into typical nonameric complexes with li. In the absence of li, the class II chains largely remain in aggregates, before degradation. This aggregation-prone behavior of class II is also seen with heterodimers produced in insect cells in the absence of li (28) and with class II heterodimers removed from nonameric complexes with li using low pH (29). In each of these circumstances, aggregation can be prevented by substituting an antigenic peptide for li. This suggests either a special role for binding site occupancy in folding class II so as to avoid nonspecific protein–protein interactions, or direct recognition by the binding site of target structures present on non-MHC protein aggregates.

In both transfected cells (20, 21) and lymphocytes of li-deficient mice (22–24), the cohort of class II heterodimers that does form in the absence of li shows defective transport from the ER to the Golgi complex. This is characteristically seen as the failure of the α and β chains in these serologically normal heterodimers to acquire endoglycosidase-resistant N-linked glycans, even at late times after synthesis. This transport deficiency can be ascribed to any of several events. One is the aggregation phenomenon, as even αβ heterodimers are associated with very high molecular complexes of incompletely assembled class II chains under some circumstances. However, li produced in the absence of class II also shows delayed exit from the ER (30), yet fails to demonstrate the aggregation typical of class II (26, 27). Another possible mechanism is the association of both li and class II chains with the ER–resident protein calnexin (p88, IP90) (31, 32), an interaction that is lost efficiently only upon completion of nonamer assembly.

Calnexin is an integral membrane protein of high abundance in the ER that associates with a large number of newly synthesized membrane-bound and soluble proteins (for reviews see references 33, 34). Release from calnexin coincides with protein folding and oligomer assembly, suggesting that calnexin acts as an ER chaperone. Different protein features have been described to be critical for association with calnexin. Some studies propose that calnexin interaction with newly synthesized proteins is mediated via the transmembrane region (35), whereas other reports suggest a specific role for N-linked glycans (36). Hammond and colleagues (37) have provided evidence that glucose residues on partially trimmed core glycans make a critical contribution to the interaction between nascent protein chains and calnexin. In these studies protein folding, glucose removal, and release from calnexin coincide. Concor-dant data on an important role for N-linked glycans in calnexin binding have recently been reported for TCR-α chains (38) and MHC class I molecules (39, 40). In contrast, the CD3ε subunit of the TCR complex, which binds strongly to human calnexin in the absence of other receptor polypeptides (41), lacks N-linked glycans. For this latter integral protein, Rajagopalan et al. (41) have suggested that the association with calnexin is regulated by the oligomeric state of the TCR complex. Arunachalam and Cresswell (42) have also recently reported that human class II and li chains produced in cells treated with tunicamycin, an inhibitor of core glycan addition, still show association with calnexin.

The notion of calnexin as a chaperone showing prolonged interactions with incompletely folded proteins would be in accord with the ER retention of class II and li produced independently of one another, with their association with calnexin shortly after synthesis, and with their efficient release from the ER, being related to the loss of this association upon formation of a properly assembled nonamer (31, 32). Such a model of calnexin as a chaperone involved in actively promoting class II–li subunit folding and/or association might predict that in the absence of its binding to one or more of these chains, the generation of properly folded oligomers would be inhibited or prevented. Our recent analysis of li structure and function revealed, however, that a truncated form of mouse invariant chain (residues 1–107) was quite efficient in assembling with class II and promoting ER to Golgi transport (43). Because this segment of li lacked both N-linked glycans as well as most of the luminal domain of li, it was possible that it failed to associate with calnexin, which, if true, would argue against a necessary role of the latter in these aspects of li function.

To explore this question further, we have directly examined the interactions of normal and mutant mouse li chains with calnexin and explored the role of N-linked glycans in this association. Our data reveal that such glycans play an important but not exclusive role in regulating li–calnexin interaction, and that li–calnexin association is not essential for class II–li assembly or transport. Rather, a major function of calnexin seems to be the retention of li in the ER and the inhibition of degradation of this retained protein. Both of these features promote effective class II–li assembly while protecting the cell from the untoward consequences of free li in the endocytic pathway (7, 44, 45).

Materials and Methods

Plasmid Construction. cDNA expression vectors containing inserts coding for wild-type mouse (m) li31, mli19-215, mli1-107, and mli19-107 have been previously described (18, 43). A cDNA expression construct containing IP90 (46) was kindly provided by Dr. M. B. Brenner (Harvard Medical School, Boston, MA).

A DNA fragment containing mli1-131, 5′ translation control elements, and EcoRI, BamHI, and HindIII cloning sites was generated by PCR amplification as previously described (43). pcEXV3ml131 was used as template, and the following oligonucleotide primers were used: upstream 5′CCGAAATTCAGGTTACTGAGGCTAGAGCCATG 3′ and downstream 5′GAGGCTAGACCCATG 3′ and downstream 5′AAGAATTCGAGTCCCTTACCTGCAGAGGTG 3′. The amplified fragment was digested with EcoRI and inserted into the EcoRI cloning site in the cDNA expression vector pcEXV3 (18).

A clone with the insert in the proper transcriptional orientation was selected for use. The wild-type and mutant li molecules studied here are shown schematically in Fig. 1.

Transient Expression. A modified DEAE–dextran procedure was used as previously described to transiently transfect COS 7.2
cells (7). After two washes with DME containing 10 mM Hepes, COS cells were incubated with DME/10 mM Hepes containing 400 μg/ml DEAE-dextran, 100 μM chloroquine, and DNA (1 μg of each plasmid). After 4 h at 37°C, the cells were treated with 10% DMSO in PBS for 2 min at room temperature and then incubated in complete medium. 48 h after transfection the cells were used for labeling and immunoprecipitation.

Metabolic Radiolabeling, Immunoprecipitation, and SDS-PAGE Analysis. Transfected COS cells were incubated for 1 h in methionine- and cysteine-free medium. 35S-TRANs label (methionine and cysteine) (500 μCi/ml) was then added and the cells incubated for the indicated time (from 5 to 30 min). Some samples were lysed immediately (pulse) either in 1% NP-40 or in 0.3% CHAPS. Other labeled cell samples were incubated for an additional 15 min or 2 h in medium containing an excess of cold methionine and cysteine (chase), then lysed as indicated. Where indicated, chloroquine (100 μM; Sigma Chemical Co., St. Louis, MO) and/or tunicamycin (10 μg/ml; Sigma Chemical Co.) were added to the culture medium during the prelabeling step and were maintained at these concentrations during pulse-labeling and chase incubations. Brefeldin A (5 μg/ml; Epicentre Technologies, Madison, WI) was added to appropriate samples during the chase only. The precleared lysates were immunoprecipitated using mAbs previously bound to protein G-Sepharose beads, as previously described (43). For tunicamycin-treated CBA spleen cells, additional preclearing steps were performed using Con A-Sepharose beads. The eluted samples were analyzed by SDS-PAGE in reducing conditions. In the cross-linking experiment, 200 μg/ml of the water-soluble, reducible cross-linking reagent dithiobis[succinimidylpropionate] (DSP; Pierce Chemical Co., Rockford, IL) were added to the lysing buffer.

Results

Relationship between α2 Glycosylation and Calnexin Binding. Hammond et al. (37) have proposed that glucose residues on partially trimmed N-linked glycans play a critical role in regulating calnexin interaction with membrane glycoproteins in the ER, and this hypothesis is well supported by several recent studies (38–40). Nevertheless, some published data argue that such carbohydrate residues are not absolutely required for calnexin binding, including work with human α2 (42). Fig. 2 shows that 4.5 h after synthesis, a greater fraction of the carbohydrates of class II molecules associated with a truncated form of mouse invariant chain lacking the normal N-glycosylation sites (α219-107) are resistant to endoglycosidase H digestion than those on either class II expressed in the absence of α2 or expressed in the presence of invariant chain lacking only a segment of the cytoplasmic tail (α219-215). These data are consistent with highly efficient export from the ER to the Golgi complex of the class II molecules associated with truncated α2 lacking N-glycosylation sites, a finding that might indicate a loss of calnexin-mediated retention of α2, as well as the absence of a requirement for α2-calnexin association during assembly and trafficking of α2-class II complexes. We therefore undertook to directly examine whether the two N-linked carbohydrates on mouse α2 contributed to the interaction of this molecule with calnexin. Two different approaches were used: drug inhibition of N-glycosylation (tunicamycin) and the study of deletion mutants of the luminal region of α2, some of which lacked the residues at which N-glycosylation normally occurs.

Proteins from lysates of pulse-labeled COS cells coexpressing intact α2131 and human calnexin (IP90) were immunoprecipitated with anti-invariant chain (IN-1; 47) or anti-human calnexin (AF-8; 46) mAbs. AF-8 immunoprecipitates showed a distinct band of coprecipitating α2131 (Fig. 3 A). The AF-8 mAb does not bind to the endogenous simian calnexin present at high concentration in the ER of the COS cells. Therefore, the amount of α2 coprecipitated with human calnexin by this mAb is likely to significantly underestimate the actual level of association of α2 with the total calnexin pool. Similarly, only a modest

![Figure 1](image-url)
Figure 2. The N-linked glycan-free truncation mutant ml19-107 assembled with class II shows enhanced ER to Golgi transport. COS cells expressing the class II chains Aα and Aβ alone or together with either ml19-215 or ml19-107 were metabolically labeled (30 min), then chased for 4 h. Detergent lysates were immunoprecipitated with Y3P (anti-Aα mAb), treated with endoglycosidase-H (+), then analyzed by SDS-PAGE and autoradiography. A13s indicates the position of Aβ chains with endoglycosidase H-sensitive glycans; Aar indicates the position of Aα chains with endoglycosidase H-resistant glycans.

amount of labeled calnexin is seen in the reciprocal immunoprecipitation with IN-1, due to a large pool of preexisting endogenous calnexin in these transfected cells that does not label under these conditions, and that competes with the newly synthesized, labeled human protein for binding to li.

Tunicamycin treatment of transfected COS cells, which inhibited most but not all the addition of core glycans to the overproduced nascent li protein chains, resulted in the coprecipitation of little or no nonglycosylated ml31 with calnexin (Fig. 3 B), consistent with several reports on the importance of N-linked oligosaccharide in nascent protein interactions with calnexin (36–40), but in distinction to the data of Arunachalam and Cresswell (42). We then analyzed the association of deletion mutants of li with calnexin. As shown in Fig. 3, C and D, neither ml1-107 nor ml1-131 coprecipitated with calnexin.

Given the results obtained with tunicamycin and ml31, it was surprising to find that ml1-131, which contains both of the N-glycosylation sites of ml31 and is extensively glycosylated in COS cells, did not show detectable association with calnexin under these conditions. These results suggest that the sugar moieties alone, while perhaps necessary, are not sufficient for tight binding of li to the chaperone. To examine whether the failure to observe calnexin–ml1-131 interaction under our standard cell lysis conditions reflected a weak but perhaps physiologically significant association between these molecules, pulse-labeled COS cells expressing human calnexin and either ml1-131 or ml1-107 were lysed in the presence of the reduction-sensitive cross-linking agent DSP. As shown in Fig. 4, reduction after immunoprecipitation with AF-8 demonstrated that ml1-131 was associated with calnexin at the time of cell lysis, whereas ml1-107 was not. Thus, the presence of N-linked carbohydrates on a molecule lacking the complete luminal portion of li (ml1-131) is sufficient for a less stable interaction with calnexin, whereas the absence of both the sugars and the COOH-terminal segment of li (ml1-107) leads to a lack of any detectable association. These latter results, together with the data shown in Fig. 2 on the effective assembly of class II with ml19-107, also indicate that li interaction with calnexin is not required for the formation of class II–li complexes.

Immunoprecipitation experiments using COS cells synthesizing class II α and β, ml31, and human calnexin in the presence of tunicamycin confirmed the ability of class II to associate with li in the absence of detectable calnexin binding (data not shown).

Elimination of Calnexin Association Promotes li Degradation in a Preendosomal Compartment of COS Cells. Many proteins that are part of oligomeric assemblies are rapidly degraded in the absence of their partner chains or interaction with resident chaperones. It was thus of interest to examine whether the virtual elimination of li–calnexin interactions in cells lacking class II affected the stability of li that had not reached its usual site of degradation in endosomes/lysosomes. To accomplish this, we examined the extent of degradation of wild-type li and li mutants by performing pulse–chase experiments in the presence of chloroquine...
and/or brefeldin A, to minimize any proteolysis of Ii in the endocytic pathway.

Such studies in COS cells expressing either mII31 or mII1-107 showed different degrees of stability of these two forms of Ii. The wild-type protein mII31 was found to be stable during the time frame of our experiments, as shown by the quantitative recovery of pulse-labeled molecules after a 2-h chase (Fig. 5 A). mII1-107 was much more susceptible to preendosomal degradation under the same conditions, with 50% loss of signal during this same time period (Fig. 5 C). Additional experiments showed that 75–80% of this form of Ii was degraded by 4 h, whereas mII31 was largely intact at this time (data not shown). These data indicate a half-life for mII1-107 of ~2 h under these conditions in transiently transfected COS cells producing large amounts of this protein.

Given that wild-type mII31 shows clear binding to calnexin whereas the truncation mutant mII1-107 does not, these results are consistent with the hypothesis that calnexin binding promotes the stability of newly synthesized invariant chains, just as calnexin–TCR-α chain association protects the latter from rapid prelysosomal destruction (38). This model would predict that tunicamycin should affect the rate of degradation of wild-type mII31 and of mII1-131, but not the glycan-free mII1-107. As expected, mII31 became sensitive to degradation when synthesized in cells cultured in tunicamycin (Fig. 5 A). In agreement with the cross-linking data showing calnexin–mII1-131 association, the fate of mII1-131 was also dramatically influenced by the presence of tunicamycin, with only 50–60% of the initial pool of labeled molecules remaining after the chase (Fig. 5 B), whereas loss of mII1-107 occurred at the same rate in the presence and absence of tunicamycin (Fig. 5 C). These data are in clear accord with the view that calnexin association plays a critical role in protecting Ii from nonendocytic proteolysis.

**Effects of Preventing Ii–Calnexin Interaction in Lymphoid Cells Normally Synthesizing Class II and Ii.** All the experiments described above were performed using transient overexpression in heterologous fibroblasts. Although our previous experience indicated that many properties of class II and Ii in these cells resembles that in lymphoid cells, some aspects of class II–Ii assembly and transport differ in the fibroblast and physiologic APC models. Therefore, to determine whether our observations concerning a role for calnexin in regulating preendosomal Ii degradation reflect what happens in "professional" APC, we examined the effect of tunicamycin on Ii degradation in freshly isolated lymphoid cells. CBA spleen cells were pulse labeled for 5 min and chased for 15 min in the presence or absence of tunicamycin. Ii immunoprecipitates were then analyzed by SDS-PAGE, autoradiography, and densitometry. In the absence of tunicamycin, mII31 in these cultured spleen cells showed no significant degradation during this brief chase period. In contrast, only 60% of the initially labeled pool of mII31 remained after 15 min of chase in cells treated with tunicamycin to prevent glycan addition and interfere with
Figure 6. The inhibition of Ii–calnexin interaction using tunicamycin promotes rapid Ii degradation but does not prevent assembly of the αβ Ii complexes in normal cells. Spleen cells from CBA mice were pulse labeled for 5 min and then chased for 15 min in the absence (left) or presence (right) of tunicamycin. Tunicamycin-treated lysates were precleared with Con A-Sepharose beads. An anti-Ii mAb (IN-1) or an anti-MHC class I mAb (10.2.16) was used in immunoprecipitations. Immunoprecipitates were separated by 12% SDS-PAGE. The numbers below each lane give the chase/pulse intensity ratios as a percentage, as determined by densitometric analysis. mlipl refers to the form of mli containing the alternatively spliced exon 6b.

calnexin association (Fig. 6), suggesting an even more rapid loss of Ii in the putative absence of calnexin binding than was seen in the COS system.

We also examined the association of Ii with class II under these conditions. MHC class II–associated Ii and total Ii were quantified by densitometry from 10.2.16 and IN-1 immunoprecipitates, respectively. The ratio of Ii in the 10.2.16 versus IN-1 precipitates, which indicates the proportion of the total Ii pool that is complexed with αβ heterodimers, decreased from 3.8 to 2 in the presence of tunicamycin. Total protein synthesis, including class II, was, however, also decreased nearly 50% in the treated cells, so correcting for the smaller class II pool subject to 10.2.16 precipitation, these data indicate that a treatment expected to eliminate Ii–calnexin association has little effect on the initial extent of Ii–class II assembly. These data are consistent with the results shown in Fig. 2 concerning effective assembly of class II with the truncation-mutant mli9-107 and experiments using tunicamycin-treated COS cells co-expressing class II, intact Ii, and human calnexin (data not shown).

Discussion

Ii and class II are among the large number of integral membrane proteins known to associate with the ER resident protein calnexin shortly after synthesis (31, 32). These associations are not efficiently disrupted until the complete nonameric complex of three class II αβ heterodimers on a trimeric core of Ii is formed, after which rapid exit of this complex from the ER can be detected. These data suggest that calnexin might play an important role in facilitating the assembly of the class II–Ii oligomer, in addition to retaining the component chains in the ER until proper coassembly is complete. These studies did not reveal, however, the fate of either class II or Ii in the absence of such calnexin binding. Studies in other model systems have implicated calnexin as a chaperone involved in the folding pathway for both soluble (48–50) and membrane-bound (37) proteins, whereas other investigations have provided evidence for calnexin acting primarily as a retention protein, regulating release of otherwise properly folded but incompletely assembled subunits of oligomeric proteins from the ER (41, 51). Studies of both TCR-α chains (38) and MHC class I heavy chains (39, 40), have also indicated that at least some component chains of oligomeric protein assemblies are rapidly degraded in the absence of such calnexin binding.

Our previous studies of Ii mutants lacking portions of the luminal domain raised a question about the need for calnexin binding in the function of Ii. Two truncated forms of Ii encompassing residues 1–107 or 19–107 lacked sites for addition of the N-linked glycans shown for some other proteins to be essential for association with calnexin, but nevertheless were very effective in performing the secretory pathway functions of Ii, which include assembly with class II α and β chains, enhanced formation of properly assembled αβ heterodimers, prevention of aggregation of class II chains with each other and/or other ER proteins, and efficient transport of class II dimers through the Golgi complex (43). We thus chose to examine the role of N-linked glycans in the interaction of calnexin with the invariant chain, and upon finding differential association of this chaperone with distinct forms of Ii, to assess the impact of a lack of calnexin–Ii binding on Ii assembly and assembly with class II.

Two different approaches were used: either treatment of cells with tunicamycin, an inhibitor of core oligosaccharide addition, or expression of a truncated form of Ii lacking the sites for addition of N-linked carbohydrate. Most or all measurable calnexin interaction with Ii was lost in the absence of such N-linked glycans, in agreement with the work of several investigators in other model systems (37–40) but in contrast to recent studies using human Ii (42). The differences between our experiments and this prior investigation cannot be readily ascribed to the use of distinct detergents for cell solubilization, as we have observed the same results reported here with either CHAPS or digitonin (Romagnoli, P., unpublished observations). Despite the high level of conservation of Ii structure during evolution, species differences remain a possible explanation for these apparently discrepant findings, especially given the existence of alternative forms of Ii produced by human (52) but not mouse cells. These forms have unique ER retention properties (53) that might supplant those of calnexin. This in turn could lead to a distinct physiology of interaction be-
tween Ii and this chaperone. Alternatively, the association of human calnexin with mouse Ii might be weaker than for the human–human combination.

Our experiments have also revealed that the strength of interaction of calnexin with Ii is not determined solely by these oligosaccharides. Detection of calnexin binding to the ml1-131 truncation mutant by immunoprecipitation required chemical cross-linking, despite the addition of N-linked glycans to this form of Ii. Thus, protein structural features, in this case controlled by the COOH-terminal Inil-131 truncation mutant by immunoprecipitation rather than oligosaccharides, play an important role in binding of calnexin to Ii. 

In the case of truncated Ii, the role of calnexin in maintaining Ii in the ER is less important. However, the amount of Ii retained in the ER is still significant, indicating that other factors also contribute to this process. 

Recent studies have demonstrated that the CLIP region of Ii is critical for Ii–class II interactions (43), and that the CLIP domain is required for proper association of Ii with class II. This interaction is mediated by the CLIP domain, which is a carbohydrate-binding site for Ii-class II complexes. 

The data suggest that calnexin association with Ii is important for maintaining Ii in the ER, but that other factors, such as the CLIP domain, also play a role in this process. 

Could the increased rate of degradation be an artifact arising from protein misfolding due to either absence of N-linked glycans on Ii produced in the presence of tunicamycin, or misfolding due to the removal of the bulk of the COOH-terminal segment of Ii in the case of ml1-107? The similar extent of degradation of both full-length and shorter forms of Ii, together with the recent structural data of Ii, indicates that the COOH-terminal segment is critical to the stability of Ii. 

The rate of degradation of Ii was found to be significantly higher in cells expressing full-length Ii compared to truncated Ii. This may be due to the presence of the COOH-terminal segment, which is critical for the stability of Ii. 

The data suggest that the rate of degradation of Ii is not simply a consequence of high expression levels, but rather is due to a property intrinsic to Ii. The high level of Ii expression in the absence of class II coexpression leads to the accumulation of Ii in the ER, which may be detrimental to cell survival. 

We and others have also shown that the production of high levels of Ii, especially in the absence of class II coexpression, leads to disturbances in the structure and function of early endosomal compartments. Cells expressing substantial amounts of free, full-length Ii show characteristic enlarged early endosomes and a delay in endocytic trafficking from these early compartments to late endosomes/lysosomes. It is difficult to produce stably transfected cells with high free-Ii levels, apparently due to these endocytic disturbances (unpublished observations). Although milder alterations in endosomal function may be evident in Ii-depleted cells, the role of calnexin in this process is not yet clear. 

The data suggest that calnexin association with Ii is important for maintaining Ii in the ER, but that other factors, such as the CLIP domain, also play a role in this process. 

Although roles for calnexin can thus be found in the prevention of Ii degradation and in Ii retention, we were unable to discern a necessary contribution of calnexin to effective assembly of Ii with MHC class II molecules. This was true of ml1-107 or ml19-107 in COS cells, intact Ii in tunicamycin-treated COS transfectants, and ml31 in normal spleen cells. Analysis of Ii and associated proteins in these cells showed that calnexin association with Ii is not tightly associated with calnexin, leaving the ER more susceptible to proteolytic attack when unbound to either calnexin or class II. A primary function of Ii association with calnexin may therefore be to protect the large pool of free Ii from such destruction, so that it can serve to efficiently interact with the entire cohort of newly synthesized class II.
behavior at later times in the spleen cells was complicated by the very rapid loss of li under tunicamycin treatment conditions, so that less labeled li was available for continued assembly or coexport with class II. Taken together, these data seem most consistent with calnexin playing a facilitating, but not essential, role in the contribution of li to control of class II assembly and transport in the secretory pathway. The lack of an absolute role may arise from redundancy in the function of various ER chaperones. Alternatively, calnexin might play a quantitative role that optimizes the function of proteins produced at a given biosynthetic rate, reducing the overall metabolic load on a cell while allowing vital functions requiring certain concentrations of exported proteins to be maintained. This would be consistent with the present observations that calnexin helps maximize the availability of newly synthesized li for assembly with class II while not being essential for this interaction. A similar contribution to the optimal use of components of other oligomeric protein assemblies would explain the consistent finding of calnexin association with components of the T and B cell receptors, or monomers of viral glycoproteins.

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