Calnexin Associates Exclusively with Individual CD3α and T Cell Antigen Receptor (TCR) α Proteins Containing Incompletely Trimmed Glycans That Are Not Assembled into Multisubunit TCR Complexes*

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Most T lymphocytes express on their surfaces an oligomeric protein complex consisting of clonotypic αβ polypeptides associated with invariant CD3γδε and ζ chains, designated the T cell antigen receptor (TCR) complex. Assembly and intracellular transport of nascent TCR proteins is believed to be assisted by their interaction with the molecular chaperone calnexin, which for certain molecules functions as a lectin for monoglycosylated glycans. However, as most of our knowledge about calnexin-TCR protein associations has been obtained under conditions of limited TCR assembly, the role of calnexin in the formation of nascent TCR complexes is unclear. Here, we studied the role of glucose (Glc) trimming and calnexin association in the oligomerization of TCRα and CD3βγ glycopolypeptides in murine splenic T lymphocytes, a model cell type for efficient assembly of complete TCR complexes. We show that removal of Glc residues from both CD3βγ proteins and TCRα proteins occurred prior to their association with any other TCR components and that calnexin specifically interacted with unassembled TCRα and CD3βγ proteins containing incompletely trimmed oligosaccharides. Interestingly, we found that removal of Glc residues from glycan chains was necessary for efficient association of calnexin with TCRα proteins but not with CD3βγ glycoproteins. These studies define Glc trimming and calnexin association as initial molecular events in the translation of CD3βγ and TCRα proteins, occurring coincident with or immediately after their translocation into the endoplasmic reticulum and preceding the ordered pairing of TCR chains. In addition, these data document that calnexin assembly with CD3βγ and TCRα glycoproteins involves both glycan-dependent and glycan-independent mechanisms.

Assembly of the T cell antigen receptor (TCR) complex occurs within the endoplasmic reticulum (ER) and proceeds in a highly ordered manner by: (i) formation of noncovalently associated pairs of δε and γε proteins, (ii) association of individual clonotypic α, β polypeptides with δε and γε pairs to form intermediate αδε and βγε protein complexes, (iii) rapid pairing of αδε and βγε chains and disulfide-bonding of CD3-associated αβ proteins to yield incomplete αδεβγε TCR complexes, and finally (iv) addition of ζζ homodimers to form complete, fully assembled αδεβγεζζ TCR complexes (1–4). Egress of TCR proteins from the ER is directly related to their assembly status; most unassembled TCR polypeptides and partial TCR complexes are retained within the ER. Only TCR proteins assembled into incomplete αδεβγεζζ TCR complexes or complete αδεβγεζζ TCR complexes effectively transit from the ER to the Golgi system (1).

The molecular chaperone calnexin is a nonglycosylated resident ER transmembrane protein that associates with numerous oligomeric protein complexes within the ER, including β2 and αb integrins (5), major histocompatibility class I (6, 7) and class II molecules (8), and the antigen receptors expressed on T and B lymphocytes (7, 9, 10). Regarding the TCR complex, four individual TCR proteins have been shown to associate with calnexin, including clonotypic TCRα and TCRβ polypeptides (7, 10) and CD3δ and CD3ε chains (7, 9, 11). Association between calnexin and ζ proteins has never been observed (12). While convincing evidence exists for association of calnexin with individual, unassembled TCR proteins, much less is known about the assembly of calnexin with incompletely assembled TCR complexes containing multiple TCR subunits. Indeed, the assembly status of most nascent TCR proteins associated with calnexin has not been rigorously evaluated (7, 9). Recently, West and co-workers reported that calnexin is expressed on the surfaces of immature thymocytes in association with CD3ε and CD3δε pairs (13, 14). Expression of such clonotype-independent complexes appears to be developmentally regulated as they were not detected on the surfaces of mature T cells (14). Unlike mature T cells, immature thymocytes do not efficiently assemble CD3 components into complete αδεβγεζζ TCR complexes (15), leading to the suggestion that calnexin association with partial TCR complexes is exaggerated in cell types that are deficient in formation of complete TCR complexes (14).

A growing body of evidence signifies that removal of glucose (Glc) residues from nascent oligosaccharide chains is important for initial association of glycoproteins with calnexin (10, 16, 17). Removal of Glc residues from immature GlcαManβGlcNAcβ species (Glc, glucose; Man, mannose; GlcNAc, N-acetyl glucosamine) is accomplished by the sequential action of ER glucosidase I and glucosidase II enzymes, which remove the outermost and two innermost Glc residues, respectively (18), a process referred to as Glc trimming. Recent studies by several laboratories indicate that calnexin recognizes glycan chains on nascent glycoproteins bearing monoglycosylated (GlcαManβGlcNAcβ) saccharides (10, 16–19), indicating that both glucosidase I and glucosidase II activities are necessary for formation of glycan substrates for calnexin binding (20). Oligo-
saccharide chains are not strictly required for calnexin association, however, as several nonglycosylated molecules interact stably with calnexin, including recombinant multidrug resistance P glycoprotein lacking N-linked addition sites (21) and the CD3ε subunit of the TCR complex (11).

The role of Glc trimming and calnexin association in the oligomerization of TCR proteins within the ER is poorly understood. Indeed, it is unknown at which stage(s) of TCR assembly Glc residues are removed from oligosaccharide chains on nascent TCR glycoproteins in any cell type. Regarding the role of calnexin in TCR assembly, it has been suggested that calnexin interacts with individual newly synthesized TCR proteins to facilitate their folding within the ER and to prevent their escape to the Golgi compartment (7, 9, 10, 12). Moreover, since calnexin has been shown to associate with all TCR components except βγδ, it has also been proposed that calnexin functions in the oligomerization of TCR proteins into incompletely assembled βγδεγεδε TCR complexes (9, 12). To determine at which stage(s) of TCR assembly Glc trimming and calnexin association occurs and to see if calnexin does, in fact, participate in the oligomerization of TCR subunits within the ER, we studied the role of Glc trimming and calnexin association in the assembly of TCRα and CD3ζ glycoproteins into TCR complexes in murine splenic T cells. Our studies show that removal of Glc residues from nascent glycan chains on both CD3ζ and TCRα proteins occurs prior to their association with other TCR proteins and that calnexin associates exclusively with unassembled CD3ζ and TCRα proteins containing incompletely trimmed glycans. Moreover, we demonstrate that Glc trimming is required for effective interaction of calnexin with TCRα proteins, but not with CD3ζ proteins, and report that removal of oligosaccharides does not affect the stability of existing protein complexes of calnexin and TCRα, CD3ζ proteins. The implications of these findings on our current knowledge of TCR assembly are discussed.

**EXPERIMENTAL PROCEDURES**

**Animals, Cell Preparation, and Reagents—**C57BL/6 (B6) mice 6–8 weeks of age were obtained from the Jackson Laboratory (Bar Harbor, ME). T cell receptor transgenic Vα11+ mice (22) were generously provided by Dr. Stephen Hedrick. Splenic T cells were obtained by incubation of single cell suspensions of spleen cells on tissue culture plates coated with rabbit anti-mouse immunoglobulin (tg) (Organon-Technika-Cappel, Malvern, PA) for 60 min at 37°C, followed by isolation of nonadherent cells. The resulting cell populations were typically 85–90% CD3ζ3, as determined by flow cytometry analysis. Castanospermine (Cas) was obtained from Calbiochem (La Jolla, CA) and was used at a final concentration of 100 μg/ml.

**Metabolic Labeling**—Cells, Lysis, and Immunoprecipitation—Metabolic pulse-labeling of splenic T cells was performed as described (4). Briefly, cells were incubated in methionine-free RPMI 1640 medium (Biofluids, Rockville, MD) containing 10% fetal calf serum and 1 mCi/ml [35S]methionine (Trans-35S-label) (ICN, Irvine, CA) for 30 min at 37°C in 5% CO2. In experiments using Cas, cells were preincubated with medium or Cas for 30 min at 37°C prior to metabolic labeling. The presence of Cas was maintained throughout the pulse and chase periods. Cells were lysed by solubilization in 1% digitonin (Wako, Kyoto, Japan) lysis buffer (20 mM Tris, 150 mM NaCl, plus protease inhibitors) at 1 × 106 cells/ml for 25 min at 4°C. Cell lysates were clarified by centrifugation to remove insoluble material. Sequential immunoprecipitation and release/recapture procedures were performed as described previously (4).

**Antibodies**—The following monoclonal antibodies (mAbs) were used in this study: I-A5-2C11, which is specific for CD3ζ proteins associated with CD3ζ or CD3ζ chains (23); HMT3.1, which recognizes unassembled and assembled CD3ζ proteins (24); H28-710, which is specific for TCRβ (25); H75-597, which is specific for TCRβ (26), and RR8-1 anti-TCR Vα11 mAb (Pharmpigen). The following antisera were used: R9 anti-CD3ζ Ab (27) and SPA-860 anti-calnexin Ab (Stressgen Biotechnologies Corp., Victoria, British Columbia, Canada).

**Glycosidase Digestions and Gel Electrophoresis—**Digestion with Endo-glycosidase H (Endo H) was performed by resuspending precipitates in glycosidase digestion buffer (75 mM sodium phosphate, pH 6.1, 75 mM EDTA, 0.1% Nonidet P-40) containing 10 milliliters of Endo H (Genzyme, Cambridge, MA); digestion with jack bean mannosidase (JB) (Oxford Glycosystems, Rosedale, NY) was performed according to the manufacturer’s instructions. Samples were placed at 37°C for 12–16 h and digestions stopped by addition of 3 × electrophoresis sample buffer. For experiments examining the effects of oligosaccharide release on association of TCR glycoproteins with calnexin, lysates were precipitated with anti-CD3ζ Abs; precipitates were either mock-treated or digested with Endo H, washed, boiled in 1% SDS, and TCR proteins recaptured with anti-TCR Abs as described above. SDS-PAGE gel electrophoresis was performed as described previously (15).

**RESULTS**

**Differential Glucose Trimming of Individual Glycan Chains on CD3ζ Proteins**—In our initial studies, the Glc trimming status of nascent CD3ζ glycoproteins synthesized in splenic T cells was examined. For these experiments, splenic T cells were metabolically pulse-labeled for 30 min with [35S]methionine, solubilized in 1% digitonin, and lysates immunoprecipitated with anti-CD3ζ Ab. Radiolabeled TCRα, CD3ζ, CD3γ, and γ chains coprecipitated with these chains in splenic T cell lysates (Fig. 1, left-hand side), reflecting their assembly into TCR complexes. For specific isolation of CD3ζ proteins, precipitates were boiled in 1% SDS, Nonidet P-40 detergent added, and released material immunoprecipitated with anti-CD3ζ Ab to recapture CD3ζ chains (4). The Glc trimming status of glycans chains on CD3ζ proteins was evaluated by digestion with jack bean mannosidase (JB), which removes eight mannoside residues from fully trimmed (Manα2→3Manα2→6GlcNAc2) oligosaccharides, but only five mannosides from incompletely trimmed (Glc3α2→Manα2→6GlcNAc2) glycan chains (17). Because CD3ζ proteins are modified by addition of 3 N-linked sugar chains, 4 glycosforms of CD3ζ can exist, reflecting the presence of 0–3 incompletely trimmed saccharide chains on CD3ζ proteins. Indeed, as shown in Fig. 1, JB digestion of CD3ζ proteins revealed the existence of one minor (A) and three major (B–D) CD3ζ glycosforms in splenic T cell lysates (Fig. 1, middle), denoting CD3ζ proteins with 3 (A), 2 (B), 1 (C), and 0 (D) incompletely trimmed glycan chains, respectively. In contrast, digestion with Endo H, which removes all but a single GlcNAc residue from both incompletely and fully trimmed glycan chains, generated a single CD3ζε species, which migrated just below the D glycoform (Fig. 1, CD3ζ EH). Existence of CD3ζ glycosforms in splenic T cells was dependent on glucosidase activity, as they were not present in lysates of splenic T cells.
Glucose Trimming, Calnexin Association, and TCR Assembly

Fig. 2. Calnexin associates specifically with unassembled CD3ε proteins containing incompletely trimmed glycan chains. A, digitonin lysates of radiolabeled splenic T cells were immunoprecipitated with anti-CD3ε Ab (left-hand side) or with anti-calnexin Ab (right-hand side). Precipitated material was released by boiling in SDS, CD3ε proteins recaptured by precipitation with anti-CD3ε Ab, and precipitated with calnexin, lysates of radiolabeled splenic T cells were sequentially immunoprecipitated with: (i) anti-CD3ε Ab followed by anti-CD3δ Ab, or (ii) anti-CD3δ Ab followed by anti-CD3ε Ab, or (iii) anti-CD3ε Ab, followed by anti-CD3β Ab, and precipitates digested with glycosidases as indicated. The positions of CD3ε glycoforms (A–D) and Endo H-sensitive CD3ε glycan chains (CD3ε EH₄) are marked. B, digitonin lysates of splenic T cells metabolically pulse-labeled for 30 min were immunoprecipitated with anti-CD3δ Ab, or sequentially precipitated with anti-TCRβ Ab, followed by anti-CD3ε mAb, and finally with anti-CD3δ Ab. Material was released from precipitates by boiling in SDS, CD3ε proteins were recaptured by precipitation with anti-CD3δ Ab, and precipitates digested with glycosidases as indicated. The positions of CD3ε glycoforms (A–D) and Endo H-sensitive CD3ε glycoproteins (CD3ε EH₄) are marked.

Cells treated with the glucosidase inhibitor castanospermine (18) (Fig. 1, right-hand side). Taken together, these results show that distinct glycoforms of CD3δ proteins are generated in splenic T cells that reflect the differential removal of Glc residues from individual glycan chains.

Calnexin Associates Specifically with Unassembled CD3δ Proteins Containing Incompletely Trimmed Glycan Chains—To determine the Glc trimming status of CD3δ proteins associated with calnexin, lysates of radiolabeled splenic T cells were precipitated with anti-calnexin Ab, precipitated material was released by boiling, and CD3δ proteins recaptured by precipitation with anti-CD3δ Ab. CD3δ proteins were digested with J B enzymes. As shown in Fig. 2A, CD3δ proteins associated with calnexin in splenic T cell lysates contained incompletely trimmed glycan chains, with most existing in the B glycoform, and to a lesser extent, the C glycoform (Fig. 2A, right-hand side).

To determine if calnexin remained associated with CD3δ proteins following their assembly with other TCR proteins and to ascertain at which stage(s) of TCR assembly Glc residues are completely removed from CD3δ proteins, a series of sequential immunoprecipitation studies was performed to examine the Glc trimming status of CD3δ proteins containing incompletely trimmed and fully trimmed glycan chains. For these experiments, digitonin lysates of radiolabeled splenic T cells were precipitated with anti-CD3δ antiserum to capture total CD3δ proteins, or were sequentially immunoprecipitated with: (i) anti-TCRβ mAb to isolate CD3δ proteins existing in complete aβδεγζ TCR and incomplete aβδεγε TCR complexes, followed by (ii) anti-CD3ε Ab to capture CD3δ proteins existing in intermediate αδεγε and partial αδεγε protein complexes, and finally (iii) anti-CD3β Ab to isolate free, unassembled CD3δ proteins. CD3δ glycoproteins were recaptured from precipitates and their Glc trimming status evaluated by J B digestion. As previously established, four distinct glycoforms of CD3δ chains existed in splenic T cell lysates (Fig. 2B, left-hand side, A–D). Clearly, both incompletely trimmed and fully trimmed CD3δ glycoforms were present in the pool of unassembled CD3δ proteins (Fig. 2B, right-hand side, A–D). In contrast, CD3δ proteins associated with CD3ε and TCRβ molecules contained glycan chains that were devoid of Glc residues as they were completely susceptible to J B digestion (Fig. 2B, middle, D). Chase studies showed that Glc residues were completely removed from CD3δ glycoproteins within several hours of their synthesis in splenic T cells, which correlated with their assembly into TCR complexes and transport to the Golgi system (data not shown).

Taken together, these results demonstrate that calnexin associates with unassembled CD3δ proteins containing incompletely trimmed oligosaccharide chains. Moreover, since CD3δ proteins assembled with CD3ε containing complexes contained fully trimmed glycan chains, these studies indicate that Glc trimming of CD3δ glycoproteins occurs prior to their assembly with other TCR proteins.

To confirm these results, we wished to assess the Glc trimming status of CD3δ proteins at an earlier time point prior to their association with CD3ε and TCRβ proteins. For these studies, splenic T cells were pulse-labeled for only 5 min and chased for 10 and 20 min. Sequential precipitations of assembled and unassembled CD3δ chains and release/recapture procedures were performed as described in Fig. 2B. As expected, the vast majority of CD3δ chains remaining at the end of the short pulse period were not associated with CD3ε chains, but existed as free, unassembled CD3δ chains (Fig. 3, top). During the chase period, nascent CD3δ proteins assembled with CD3ε chains (Fig. 3, middle), and successively with TCRβ molecules (Fig. 3, bottom). These results show that the first detectable
CD3α proteins assembled with other TCR proteins in our studies contained fully trimmed glycan chains. Note that most nascent CD3α proteins synthesized during a 5 min pulse period were susceptible to J b digestion (Fig. 3, top, A–D), indicating that processing of nascent CD3α proteins by ER glucosidase enzymes occurs coincident with or immediately after their translation and insertion into the ER lumen (28, 29).

Calnexin Associates with Unassembled TCRα Proteins Containing Incompletely Trimmed Oligosaccharides—Next, we examined the Glc trimming status of TCRα proteins associated with calnexin and CD3 proteins in splenic T cells. Since the TCRα protein is clonotypic and thus quite heterogeneous, transgenic mice expressing a single rearranged TCRα gene product, the Vα11 protein, were used for these experiments (22). Splenic T cells of Vα11 transgenic mice were pulse-labeled for 30 min, solubilized in 1% digitonin, and lysates were sequentially precipitated with anti-CD3 mAb to isolate CD3-associated TCRα proteins, followed by anti-Vα11 mAb to capture unassembled TCRα proteins. Precipitated material was released by boiling in SDS, TCRα proteins were digested with J B or Endo H glycosidases. Unassembled TCRα proteins existed in two major glycoforms in splenic T cell lysates, marked A and B, which represent “free” TCRα proteins containing incompletely trimmed and fully trimmed glycan chains, respectively (Fig. 4, right-hand side). As demonstrated, TCRα proteins associated with calnexin contained incompletely trimmed glycans (Fig. 4, left-hand side, A), whereas TCRα proteins assembled with CD3α chains possessed fully trimmed oligosaccharide chains (Fig. 4, middle, B). Thus, similar to what was observed for CD3α proteins, these results show that calnexin associated specifically with unassembled TCRα proteins containing incompletely trimmed N-linked sugar chains, and that Glc residues were removed from glycan chains on nascent TCRα proteins prior to their assembly with CD3α proteins. Although transgenic mice were used in these experiments, it should be realized that similar conclusions were reached in studies using normal, nontransgenic mice (data not shown). It should also be appreciated that radiolabeled TCRβ proteins are not visible in these studies as formation of CD3-associated disulfide-linked αβ heterodimers involves pairing of newly synthesized TCRα proteins with pre-existing nonradiolabeled TCRβ proteins in murine T cells (15).

Requirement for Oligosaccharide Chains for Association of CD3α Proteins with Calnexin—To assess the importance of Glc trimming in the initial association of nascent TCRα and CD3α proteins with calnexin, studies were performed on splenic T cells treated with the glucosidase inhibitor Cas. As shown in Fig. 5A, Glc trimming was required for efficient association of nascent TCRα proteins with calnexin as only trace amounts (approximately 10%) of TCRα proteins were recaptured from anti-calnexin precipitates of Cas-treated splenic T cells relative to untreated splenic T cells (Fig. 5A, top). Surprisingly, we found that association of calnexin with CD3α chains was only modestly affected by blockade of glucosidase activity, as significant amounts of CD3α proteins were associated with calnexin in Cas-treated splenic T cells (Fig. 5A, bottom). These data indicate that Glc trimming is required for effective interaction of TCRα proteins, but not CD3α proteins, with calnexin in splenic T cells.

We reasoned that CD3α proteins might associate directly with calnexin molecules in Cas-treated splenic T cells, or indirectly, via pairing with CD3α proteins (11). To examine this issue, we evaluated the ability of various anti-CD3α mAbs to preclude calnexin-associated CD3α proteins from splenic T cell lysates. As demonstrated in Fig. 5B, precipitation of Cas-treated splenic T cell lysates with anti-CD3α Abs effectively removed calnexin-associated CD3α chains, as expected (Fig. 5B, compare lanes 1 and 4). In contrast, precipitation with two different anti-CD3 mAbs failed to preclude calnexin-associated CD3α proteins from lysates of Cas-treated splenic T cells (Fig. 5B, compare lanes 1, 2, and 3). Thus, we conclude that CD3α proteins associate directly with calnexin molecules in Cas-
chains are important in the initial assembly of TCR and CD3γδ glycoproteins. Digoxin lysates of radiolabeled splenic T cells were immunoprecipitated with anti-calnexin Ab; precipitates were resuspended in Endo H digestion buffer in the presence or absence of Endo H and incubated at 37°C for 4 h. Following digestion, precipitates were washed in PBS; supernatants containing material released by Endo H digestion were removed, pellets (precipitates) were boiled in SDS, and TCR proteins recaptured by precipitation with anti-TCR specific Abs. The positions of CD3δ and TCRα proteins are indicated. Note that negligible amounts of TCRα and CD3δ proteins were recovered from supernatants of mock-treated and Endo H-digested calnexin precipitates (data not shown).

Fig. 6. Oligosaccharide chains are not necessary to maintain stable association of calnexin with TCRα and CD3γδ glycoproteins. Digoxin lysates of radiolabeled splenic T cells were immunoprecipitated with anti-calnexin Ab; precipitates were resuspended in Endo H digestion buffer in the presence or absence of Endo H and incubated at 37°C for 34 h. Following digestion, precipitates were washed in PBS; supernatants containing material released by Endo H digestion were removed, pellets (precipitates) were boiled in SDS, and TCR proteins recaptured by precipitation with anti-TCR specific Abs. The positions of CD3δ and TCRα proteins are indicated. Note that negligible amounts of TCRα and CD3δ proteins were recovered from supernatants of mock-treated and Endo H-digested calnexin precipitates (data not shown).

Treatment of splenic T cells. While these experiments do not formally exclude the possibility that anti-CD3ε mAbs do not effectively recognize calnexin-associated δε protein complexes in Cas-treated splenic T cells, it should be noted that these mAbs are capable of precipitating calnexin-associated CD3δε proteins formed in immature T cells (14).

Finally, we wished to evaluate whether N-linked glycan chains on TCRα and CD3δε glycoproteins were required for maintaining stable interaction with calnexin. Calnexin precipitates of radiolabeled splenic T cell lysates were either mock-treated or digested with Endo H, and association of TCR glycoproteins with calnexin before and after deglycosylation was compared. As demonstrated, equivalent amounts of TCRα and CD3δε proteins were recaptured from mock-treated and Endo H-digested anti-calnexin precipitates (Fig. 6), showing that once formed, complexes of calnexin and individual CD3δε and TCRα proteins do not require oligosaccharide chains to maintain their association.

DISCUSSION

The quality control system of the ER ensures that properly folded, fully assembled protein complexes are expressed on the cell surface. The molecular chaperone calnexin is believed to assist in the oligomerization of nascent TCR proteins within the ER and to play a role in regulating the transport of assembled TCR complexes from the ER to the Golgi compartment. In the current study, we evaluated the role of Glc trimming and calnexin association in the oligomerization of CD3δε and TCRα proteins in splenic T lymphocytes. Our results show that: (i) removal of Glc residues from nascent CD3δε proteins and TCRα proteins occurs prior to their association with partner TCR proteins within the ER; (ii) calnexin associates specifically with unassembled CD3δε proteins and unassembled TCRα proteins containing incompletely trimmed glycan chains; (iii) Glc trimming is required for effective association of TCRα proteins, but not CD3δε proteins, with calnexin; and (iv) oligosaccharide chains are important in the initial assembly of TCRα-calnexin protein complexes, but that once formed, are not required to maintain their association. Taken together, these data effectively rule out the postulate that calnexin functions as a scaffold for assembly of nascent TCR complexes and show that calnexin assembly with TCR glycoproteins involves both glycan-dependent and glycan-independent mechanisms.

Recent studies suggest that association of nascent glycoproteins with calnexin proceeds in a two-step fashion involving

initial binding of monoglycosylated glycans by calnexin, followed by protein-protein interactions which stabilize these associations (19, 30). In agreement with this model, we found that inhibition of glucose trimming markedly impaired association of nascent TCRα proteins with calnexin in splenic T cells and, similar to what has been reported for major histocompatibility class I proteins (19) and major histocompatibility class II proteins (31), removal of oligosaccharide chains from existing calnexin-TCRα complexes did not affect the stability of their interaction. Interestingly, the transmembrane domain of all of these proteins has been implicated in maintaining their association with calnexin (31, 32), suggesting that calnexin interactions may be stabilized within the lipid bilayer of the ER. The finding that blockade of Glc trimming severely limits the assembly of nascent TCRα proteins with calnexin in splenic T cells is in agreement with previous studies showing that association of nascent TCRα proteins with calnexin is impaired in glucosidase II-deficient BW PHAR2.7 thymoma cells and in Cas-treated wild type BW thymoma cells (10). Importantly, the current study extends these findings by showing that, unlike calnexin association with TCRα proteins, assembly of calnexin with CD3δε proteins was only modestly affected by inhibition of Glc trimming. Thus, CD3δε represents the second TCR component that has been described to interact with calnexin in a glycan-independent manner, with CD3ε being the first (11). Because CD3δε and CD3ε proteins are structurally homologous (1), it is reasonable to speculate that CD3δε and CD3ε molecules share a common region that mediates their association with calnexin. It remains to be determined why some CD3δε chains synthesized in Cas-treated splenic T cells failed to associate with calnexin in our studies. The presence of monoglycosylated glycans on CD3δε proteins, although not absolutely required, may increase the efficiency with which CD3δε proteins interact with calnexin. Alternatively, as persistence of Glc residues on oligosaccharide chains has been observed to result in increased association of CD3δε chains with CD3ε molecules (4), it is conceivable that enhanced assembly of CD3δε chains into δε pairs in Cas-treated splenic T cells precludes their association with calnexin.

The role of calnexin in the oligomerization of TCR proteins has been controversial as most studies on calnexin association with TCR proteins have been performed in cell types that are markedly impaired or completely deficient in the assembly of complete αβδεεγγ γεγεγγ γγγ γγγ γγγ γγγ γγγ γγγ γεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγεγ
facilitates displacement of calnexin; importantly, however, our data show that calnexin does not remain associated with TCRα and CD3δ proteins assembled into multisubunit TCR complexes in splenic T cells. Finally, it is noteworthy to mention that our results on calnexin association with unassembled TCR components in splenic T cells parallel recent findings on the assembly of IgM complexes in B cells, which showed that calnexin associated with free IgM heavy chains but not with IgM chains assembled with Igα proteins (33).

In summary, the current report has evaluated the role of Glc trimming and calnexin association in the assembly of CD3δ and TCRα proteins into TCR complexes in splenic T cells. These studies define Glc trimming and calnexin association as initial molecular events in the assembly cascade of TCR glycoproteins that precede the ordered pairing of TCR chains within the ER.

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