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Endoplasmic reticulum retention and degradation of T cell antigen receptor β chain

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Abbreviations: ER, endoplasmic reticulum; DMEM, Dulbecco's modified Eagle's medium; PMSF, phenylmethylsulfonyl fluoride; TCR, T cell antigen receptor

Abstract

The T cell antigen receptor-CD3 (TCR/CD3) complex is assembled in the endoplasmic reticulum (ER) of T cells after synthesis of individual chains, and is transported to the cell surface for immune recognition and regulation. Partially assembled or unassembled TCR chains are retained and rapidly degraded in the ER. These processes are strictly regulated in the ER at post-translational level for the maintenance of cellular homeostasis. In order to identify the region responsible for the ER retention and rapid degradation of the TCR β chain, number of mutants were engineered and their fates, after synthesis in the ER of the HeLa cells, were investigated. Extensive mutagenic analysis of TCR β chain, including changing the charged amino acid residues and two tyrosine residues of the transmembrane region into hydrophobic amino acid residues, did not alter the ER retention and rapid degradation. Soluble TCR β chain and cytoplasmic tail truncation mutant were also rapidly degraded in the ER. However, N-glycosylation rate of soluble TCR β chain in the ER was significantly increased possibily due to the increased exposure of the N-glycosylation site. These results suggest that the ER retention of TCR β chain is mediated through its extracellular and transmembrane-cytoplasmic regions and that the rapid ER degradation can be caused by an exposure of unassembled subregion of TCR β chain, either extracellular domain or hydrophobic transmembrane region to the hydrophilic environment (lumen of the ER) rather than by presence of a specific degradation signal.

Keywords: endoplasmic reticulum (ER), T cell antigen receptor (TCR)

Introduction

The endoplasmic reticulum (ER), is a site of secretory, membrane, lysosomal, and vacuolar protein synthesis. Newly synthesized proteins are translocated into the lumen of the ER where initial modification occurs: cleavage of signal sequence, N-linked glycosylation, disulfide bond formation, correct folding of monomeric proteins and oligomerization of polypeptide subunit (Braakman *et al*., 1992; Gething and Sambrook, 1992; Hwang *et al*., 1992; De Silva *et al.,* 1993). Properly folded and oligomerized proteins are further modified in the Golgi area and vesicular transported to their predestined place, e.g. cell surface or extracellular secretion (Pfeffer and Rothman, 1987; Lodish, 1988). In contrast, ER resident proteins, both luminal ER soluble proteins and transmembrane ER proteins, escape from the default bulk flow transport by the presence of specific retention signals. Various molecular signals necessary for the ER retention of the nascent proteins have been identified. Soluble ER luminal and transmembrane ER proteins have peptide sequence, responsible for ER retention, at their C-terminal ends (Munro and Pelham, 1987; Jackson *et al*., 1990; Shin *et al*., 1991; Gomard *et al*., 1997). Newly synthesized proteins are screened by ER resident proteins, immunoglobulin binding proteins (BiP). Misfolded poly-peptides and unassembled subunits of oligomeric proteins are captured by BiP, retained in the ER, and rapidly degraded in the lumen of the ER (Bonifacino *et al*., 1989; Klausner and Sitia, 1990; Sitia *et al*., 1990; Wileman *et al*., 1990; Wikstorm and Lodish, 1992; Beggah *et al*., 1996). This rapid and selective degradation of proteins have been described as pre-Golgi or ER degradation (Lippincoctt-Scchwartz *et al*., 1988). Some of the best characterized examples of this type comes from the TCR biosynthesis studies.

TCR is a disulfide heterodimer (αβ or γδ) intimately associated with at least five non-polymorphic polypeptide components of the CD3 molecule (γ, δ, ε, ζ and η) at the cell surface (Clevers *et al*., 1988; Klausner *et al*., 1990). The TCR/CD3 complex is assembled in the ER of T cells after synthesis of individual chains, further modified in the Golgi apparatus and transported to the cell surface (Carson *et al*., 1991). Partially assembled or unassembled TCR/CD3 components are retained and/or degraded in Bonifaino, 1991). In cells where TCR/CD3 components are severly imbalanced, such as precursor T cells, leukemic T cells, or cDNA transfected Cos cells, the murine TCR α, β chain and CD3 γ, δ subunits are rapidly degraded in a non-lysosomal preGolgi compartment (ER degradation). Tight control of proper folding and complete assembling of TCR/CD3 complex, which result

in cell surface expres-sion, requires intra- and inter-molecular informations for sorting and trafficking molecules from one organelle to another. Sorting and trafficking single peptide, e.g for ER retention or for transport from ER to lysosome or from cytoplasm to nucleus, is strictly regulated by information intrinsically present in the polypeptide sequences (Dang and Lee, 1989; Baranski *et al*., 1990). Cell surface expression of TCR/CD3 complex and degradation of partially assembled or excessively synthesized TCR sub-units are strictly regulated in the ER at post-translational level. These processes are essential for the maintenance of cellular homeostasis and quality control of newly synthesized proteins in the cell. Although the fate of partially assembled TCR/CD3 complex has been revealed to some extent, the molecular information that regulates this process is not well understood. Transmembrane domains of TCR α and β chain have been proposed to hold such information. Furthermore, one or two positively charged amino acids residues present within these transmembrane regions have been suggested to regulate ER retention and degradation of the TCR chains as well as assembly with CD3 components (Bonifacino *et al*., 1990a,b; Manolios *et al*., 1990)

In this study, a number of TCR β mutants were engineered and their fates were followered to investigate whether the molecular information embedded in the extracellular, transmembrane and cytoplasmic regions of the TCR β chain affects the ER retention and degradation. The results of these experiment suggest that the rapid degradation of unassembled TCR β chain in the ER may be caused by the unstable conformation and that the ER retention may be mediated by the extracellular, transmembrane and cytoplasmic domains rather than by the presence of specific retention signal.

Materials and Methods

Materials

Dulbecco's modified Eagle's medium (DMEM), DME methionine free medium, leupeptin, phenylmethylsulfonyl fluoride (PMSF) and anti-mouse IgG antibody were purchased from Sigma Co (St. Louis, U.S.A). T4 DNA ligase and other restriction enzymes, endoglycosidase H (Endo H), protein A-agarose were obtained from Boehringer Mannheim Biochemica (Germany). [^{35}S]dATP and [^{35}S]Methionine were purchased from Amersham Co (Buckinghamshire, UK). M13 Muta-Gene Kit and electrophoresis reagents were purchased from Bio-Rad Co (Hercules, CA, U.S.A). βF1 monoclonal antibody were purchased from T Cell Science (Cambridge, MA). X-OMAT imaging film and 3-H Enhancer solution were purchased from Kodak and Dupont Co (Boston, MA). Other chemical reagents were of analytical grade.

Mutagenesis, transfection and metabolic labelling

Oligonucleotide site-directed mutagenesis was performed in human cytotoxic TCR β cDNA (Leiden *et al*., 1986) containing single stranded bacteriophage M13 (Kunkel, 1985). Mutagenized TCR β inserts were subcloned into the expression vector pcDNA1 (Invitrogen Co. San Diego, CA) for eukaryotic expression. HeLa cells were maintained in 10% fetal calf serum supplemented with DMEM and transiently transfected with 25 μg of cDNA per 10-cm dish using the calcium phosphate precipitation method (Chen and Okayama, 1987). HeLa cells expressing the TCR β chains between 48-72 h after transfection were metabolically labelled with [^{35}S]methionine (150 μCi/ml) in methionine-free DME medium for 30 min and their fates were chased in complete DMEM for 0 to 8 h.

Immunoprecipitation and endo H digestion

Cells were lysed for 10 min in 1×10^7 cells per 1 ml of lysis buffer (10 mM Tris, pH 7.4, 150 mM NaCl, 1% Triton X 100, 1 mM PMSF, 1 mg/ml leupeptin). Insoluble material was removed from the lysate by centrifuging at 15,000 rpm for 20 min at 4˚C. Lysates were precleared for one hour with Anti-mouse IgG antibody and protein A-agarose and centrifuged at 15,000 rpm for 2 min. βF1 monoclonal antibody and protein A-agarose were added to the supernatant lysate and incubated for 2 h. The immunoprcipitates were eluted from antibody-protein A agarose conjugate by boiling in the presence of 1% SDS in 10 mM sodium phosphate, dissolved in 2$\times$ volume of sodium phosphate buffer (pH 7.0) and digested with 1 mU of endo H for overnight at 37˚C. Samples were analyzed on 10% SDS-PAGE, fluorographed and exposed to X-Omat X-ray film.

Results

HeLa cells expressing the TCR β chain and its mutants were metabolically labelled with [^{35}S]methionine and their fates were chased. The wild type TCR β chain, as controls, was retained and rapidly degraded in the ER. Endo H digestion of this molecule produced single band that represents the backbone polypeptide, thus providing evidence for the ER retention and degradation (Figure 1). The two positively charged amino acids in the transmembrane region of the TCR α chain have been suggested to be a specific motif for ER retention and degradation. It has been also suggested that these charged amino acids are recognized in the lipid bilayer, resulting in rapid degradation in or near the ER (Bonifacino *et al*., 1990a, b; Manolios *et al*., 1990). To identfy the molecular information embeded in the transmembrane regions of the TCR β chain responsible for ER retention and degradation,

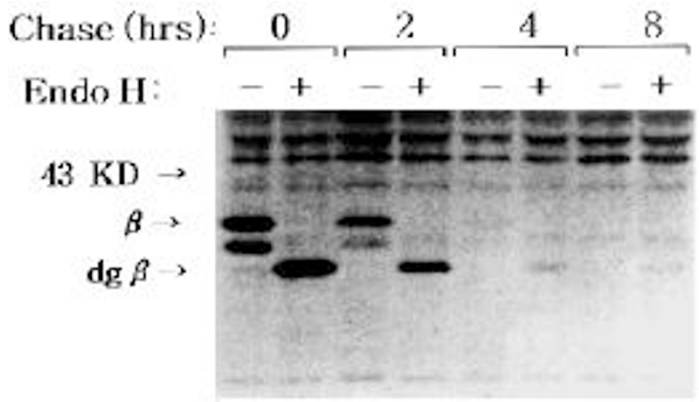

Figure 1. Fates of TCR β chain. Hela cells expressing the TCR β chain were metabolically labelled with [^{35}S]methionine for 30 min at 37˚C, after which they were placed in regular culture medium at 37˚C for 0, 2, 4, 8 h, as indicated. Labelled TCR β chain was isolated by immunoprecipitation using a βF1 monoclonal antibody. Immunoprecipitates were either not treated (-) or treated with endo H (+) prior to the SDS-polyacrylamide gel electrophoresis. The size marker, N-glycosylated form (β) and deglycosylated form (dg β) of TCR β chain are marked by arrows.

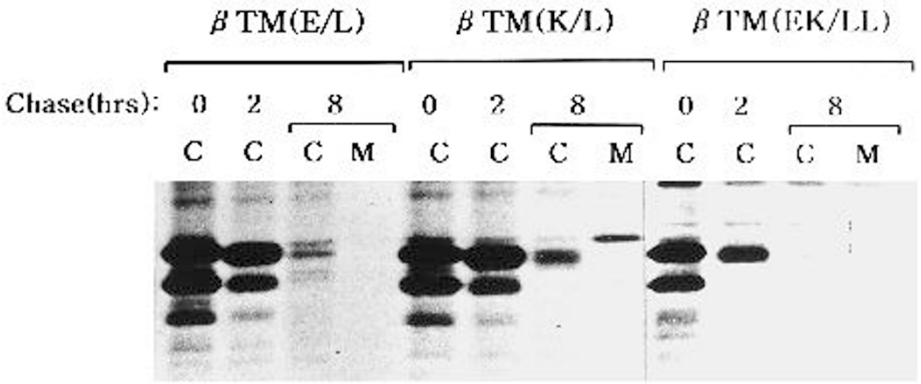

Figure 2. Effects of charged amino acid residues of transmembrane region on the fate of TCR β chain. The two charged amino acid residues (E262/L, K267/L) of transmembrane region were mutated to leucine, individually or both. The cell lysate (C) and culture medium (M) were immunoprecipitated and their fates were chased as previously described.

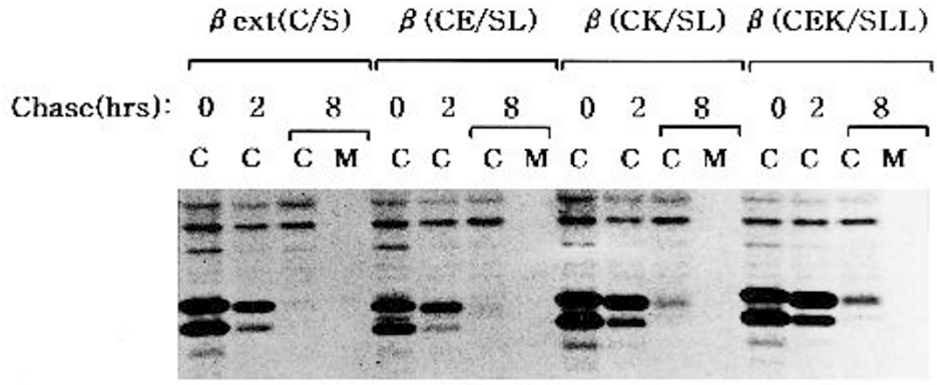

Figure 3. Effects of cysteine residues of extracellular domain and charged amino acid residues of transmembrane region on the fate of TCR β chain. The cysteine residue(C243/S) of extracellular domain was mutated to serine and two charged amino acid residues (E262/L, K267/L) of transmembrane region were mutated to leucine, seperately or simultaneously. The cell lysate (C) and culture medium (M) were immunoprecipitated and their fates were followed as previously described.

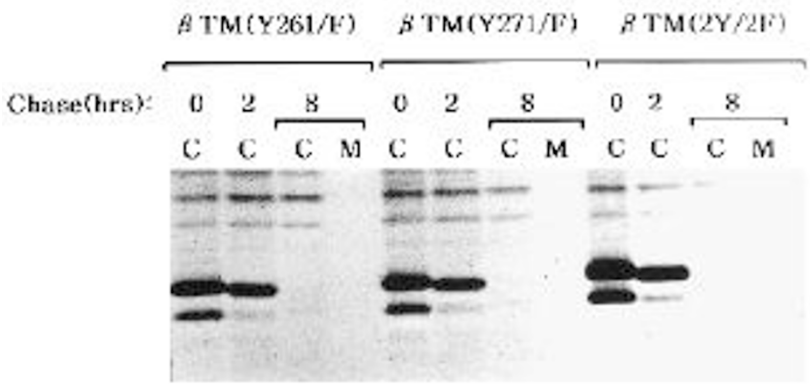

Figure 4. Effects of tyrosine residues of transmembrane region on the fate of TCR β chain. The two tyrosine residues (Y261/F, Y271/F) of transmembrane region were mutated to phenylalanine, seperately or both. The cell lysate (C) and culture medium (M) were immunoprecipitated and their fates were chased as previously described.

the negatively and positively charged amino acid residues (E262/L, K267/L) of the putative transmembrane region were mutated to leucine, seperately or simultaneously. All of these TCR β chain mutants were rapidly degraded in the ER without retention or secretion into culture medium (Figure 2). The cysteine residue (C243/S) of extracellular domain (disulfide bond formation with TCR α chain) and the two charged amino acid reidues (E262/L, K267/L) of transmembrane region were mutated to serine and lysine, seperately or simultaneously. These mutants followed almost the same fate as the wild type TCR β chain (Figure 3). Tyrosine residues of transmembrane and cytoplasmic domain in cell surface molecules are known to be directly involved in the endocytosis and lysosomal targeting (Williams *et al.,* 1990). The separate or simultaneous mutation of two tyrosine residues (Y261/F, Y271/F) of the transmembrane region to Phenylalanine were also rapidly degraded in the ER (Figure 4). To evaluate the subregion of TCR β chain for ER retention and degradation, the transmembrane and cytoplasmic domains of TCR β cDNA were truncated respectively, and their fates, after the transfection into HeLa cells, were investigated. Soluble TCR β chain (Q252/stop) and its mutant showed rapid degradation as similar as full length TCR β chain without ER retention or secretion into culture medium. However, N-glycosylation rate of soluble TCR β chain in the ER was significantly increased compare to that of wild type TCR β chain (Figure 5). In order to identify the role of short cytoplasmic tail (-KRKDF), which contains tramsmembrane ER protein retention signal, cytoplasmic tail was truncated and the two charged amino acid residues of transmembrane region were mutagenized to leucine, seperately or simultaneously. These cytoplasmic tail truncated mutants were also rapidly degraded in the ER and did not induce the ER retention (Figure 6).

Discussion

The newly synthesized ER resident proteins contain various retention or degradation motif at their extracellular, transmembrane domains and C-terminal ends. ER resident proteins are retained in the ER wthout being transported to Golgi apparatus by the presence of specific retention motif.

When the cDNA encoding the wild type TCR β chain was transfected into HeLa cells, more than 80% of the newly synthesized TCR β chain was retaind and rapidly degraded in the ER. TCR β chain has three N-linked glycosylation sites at extracellular domain. N-linked

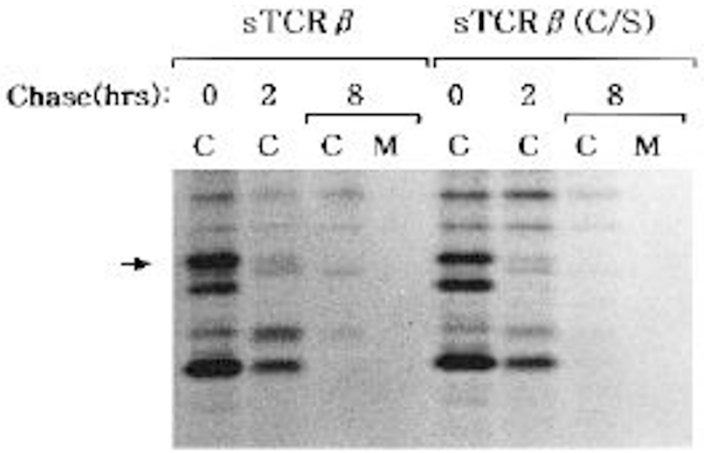

Figure 5. Fates of soluble TCR β chain. The glutamine residue (Q252/stop) of extracellular domain, just above the transmembrane region, was mutated to stop codon. Extracellular domain of cysteine residue (C243/S) of the soluble TCR β chain was mutated to serine. The cell lysate (C) and culture medium (M) were immunoprecipitated and their fates were followed as previously described. The increased N-glycosylated form of TCR β chain is marked by arrows.

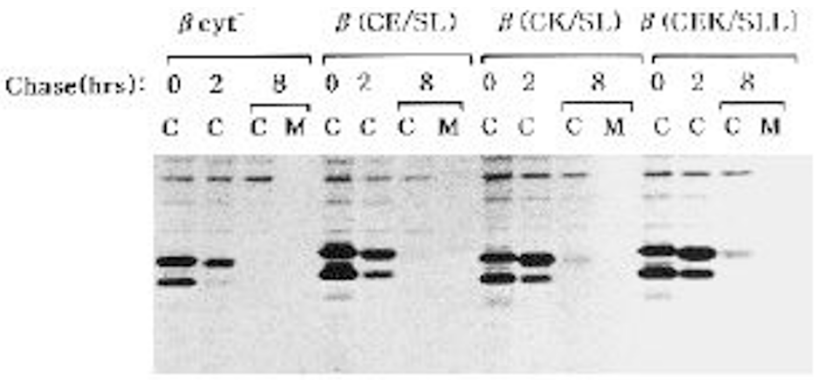

Figure 6. Fates of cytoplasmic tail truncation mutant of TCR β chain. The lysine residue (K285/stop) of cytoplasmic domain, just below the transmembrane region, was mutated to stop codon. Extracellular domain of cysteine residue (C243/S) and transmembrane region of two charged amino acid resiues (E262/L, K267/L) were mutated to leucine, seperately or simultaneously. The cell lysate (C) and culture medium (M) were immunoprecipitated and their fates were chased as previously described.

glycosylation of proteins in the ER results in addition of high mannose oligosaccharide(s) which is modified in the medial Golgi to a complex oligosaccharide (Hirschberg *et al*., 1987), no longer sensitive to digestion with endo H. TCR β chain, metabolically labelled with [^{35}S]methionine, purified by immunoprecipitation and digested with endo H, showed more rapid migration on SDS-PAGE than undigested protein. These reults suggest that the TCR β chain is retained and rapidly degraded in the ER without being transported to Golgi apparatus.

Transmembrane domains of type 1 membrane proteins are mainly composed of approximately 20 hydrophobic amino acid residues. These hydrophobic amino acid residues of transmembrane domain are assumed to form an amphiphilic α-helix and stabilized in the hydrophobic environment of lipid bilayer. The charged amino acid residues are relatively scant in the transmembrane region. The charged amino acid residues of putative transmembrane domain have been proposed to play an important roles in the assembly with other subunit, intracellular fate, and biological function.(Bonifacino *et al*., 1990a, 1991; Wileman *et al*., 1993) Interestingly, the transmembrane regions of the TCR chains contains charged amino acid residues, two positively charged amino acids in the α chain, and one negatively and one positively charged residues in the β chain. Extensive mutagenic analysis of transmembrane domain of TCR β chain, including changing the charged amino acid residues and two tyrosine residues into hydrophobic amino acids, resulted in retention and rapid degradation in the ER; these mutation did not alter the fate of TCR β chain. The length of transmembrane domain has been suggested to play a critical role in determining the intracellular fate of newly synthesized proteins(Lankford *et al*., 1993). The putative transmembrane domain of TCR β chain is composed of 30 amino acid residues. The length of transmembrane domain of TCR β chain is relatively longer than other membrane proteins and the upstream region of putative transmembrane domain contains polar and charged acids which destabilizes α-helix formation in the lipid bilayer (S256, T258, Y261, E262, G266, K267, T269, Y271). These polar and charged amino acid residues might interfere with the physiochemical stability of the transmembrane domain embeded in the lipid bilayer. The structural instability could result in the release of the transmembrane domain from the lipid bilayer and the exposure of hydrophobic residues into the hydrophilic environment of ER lumen which may lead to the rapid degradation by degradative materials. Therefore, ER retention and rapid degradation of TCR β chain may occur due to the unstable structure rather than the charged amino acid residues.

Extracellular domain of TCR α chain (soluble TCR α) has been proposed to have ER retention and degradation motif (Shin *et al*., 1993). To evaluate the subregion of TCR β chain for ER retention and degradation, transmem-brane and cytoplasmic domains of TCR β chain were truncated and its fate was investigated after the transfection into HeLA cells. Soluble TCR β chain and its mutant did not induce ER retention and/or degradation. These results are inconsistant with the soluble TCR α chain. However, N-linked glycosylation rate of soluble TCR β chain was significantly increased compared to that of the wild type TCR β chain, possibly due to the increased exposure of N-linked glycosylation site during the folding process in the ER. The length of cytoplasmic domain and its structure are also important in the induction of ER retention (Shin *et al*., 1993). Short cytoplasmic tail (6 amino acids) of TCR β chain has retention motif of ER transmembrane proteins at C-terminal end (-KRKDF). Truncation of cytoplasmic tail of TCR β chain and thier mutants did not alter the rapid degradation of TCR β chain. Since the ER retention is mediated by protein-protein interaction(Tiganos *et al*., 1997), it is important to know how far away the retention motif located from the membrane region. In the CD4 system, this retention motif was 10 amino acids distal from the membrane (Shin *et al*., 1991). However, cytoplasmic tail of the ER retention sequence in the TCR β chain is only two amino acid distal from the transmembrane region. This finding suggests that the retention sequence of TCR β chain do not directly induce ER retention.

These results suggest that the ER retention of TCR β chain is mediated through its extracellular and transmembrane-cytoplasmic regions. Rapid degradation of TCR β chain can be caused by exposure of unassembled subregion of TCR β chain, either extracellular domain or unstable structre of transmembrane region to the hydrophilic environment (lumen of the ER) rather than by the presence of a specific degradative signal. In addition, these results support our hypothesis of ER degradation taking place in the lumen of the ER due to the unstable conformation of the protein in the lumen of the ER (Shin *et al*., 1993).

References

Baranski, T. J., Faust, P. L. and Kornfeld, S. (1990) Generation of a lysosomal enzyme targeting signal in the secretory protein pepsinogen. *Cell* 63: 281-291

Beggah, A., Mathews, P., Beguin, P. and Geering, K. (1996) Degradation and endoplasmic reticulum retention of unassembled alpha- and beta- subunits of Na,K-ATPase correlate with interaction of BiP. *J. Biol. Chem.* 271: 20895-20902

Blumberg, R. S., Alarcon, B., Sancho, J., Mcdermott, F. V., Lopez, P., Breitmyer, J. and Terhorst, C. (1990) Assembly and function of the T cell antigen receptor requirement of either the lysine or arginine residues in the transmembrane region of the α chain. *J. Biol. Chem.* 265: 14036-14043

Bonifacino, J. S., Suzuki, C. K., Lippincott-Schwartz, J., Weissman, A. M. and Klausner, R. D. (1989) Pre-Golgi degradation of newly synthesized T cell antigen receptor chains: intrinsic sensitivity and the role of subunit assembly. *J. Cell. Biol.* 109: 73-83

Bonifacino, J. S.,Cosson, P. and Klausner, R. D. (1990a) Colocalized transmembrane

determinants for ER degradation and subunit assembly explain the intracellular fate of TCR chains. *Cell* 63: 503-513

Bonifacino, J. S., Suzuki, C. K. and Klausner, R. D. (1990b) A peptide sequence confers retention and rapid degradation in the endoplasmic reticulum. *Science* 247: 79-82

Bonifacino, J. S.,Cosson, P. Shah, N. and Klausner, R. D. (1991) Colo-calized transmembrane determinants for ER degradation and subunit assembly explain the intracellular fate of TCR chains.*EMBO J.* 10: 2783-2793

Braakman, I., Helenius, J. and Helenius, A. (1992) Role of ATP and disulphide bonds during protein folding in the endoplasmic reticulum. *Nature* 356: 260-262

Carson, G., Kuestner, R. E., Ahmed, A., Pettey, C. L. and Concino, M. F. (1991) Six chains of the human T-cell antigen receptor-CD3 complex are necessary and sufficient for processing the receptor heterodimer to the cell surface. *J. Biol. Chem.* 266: 7883-7887

Chen, C. and Okayama, H. (1987) High efficiency transformation of mammalian cells by plasmid DNA. *Mol. Cell. Biol.* 7: 2745-2752

Clevers, H., Alarcon, B., Wileman, T. and Terhorst, C. (1988) The T cell receptor/CD3 complex: a dynamic protein ensemble. *Ann. Rev. Immunol.* 6: 629-662

Dang, C. V. and Lee, W. M. F.(1989) Nuclear and nuclear targeting se-quence of c-erb, c-myc, N-myc, P53, HS70, and HIV tat proteins. *J. Biol. Chem.* 264: 18019-18023

De Silva, A., Braakman, I. and Helenius, A. (1993) Posttranslational folding of vesicular stomatitis virus G protein in the ER: involvement of noncovalent and covalent complexes. *J. Cell. Biol.* 120: 647-655

Faye, L. (1997) The C-terminal HDEL sequence is sufficient for retention of secretory proteins in the endoplasmic reticulum (ER) but promotes vacuolar targeting of proteins that escape the ER. *Plant J.* 11: 313-325

Gething, M. J. and Sambrook, J. (1992) Protein folding in the cell. *Nature* 355: 33-45

Gomord, V., Denmat, L. A., Fitchette-Laine, A. C., Satiat-Jeunemaitre, B., Hawes, C. and Hirschberg, C. B. and Snider, M. D (1987). Topography of glycosylation in the rough endoplasmic reticulum and golgi apparatus. *Ann. Rev. Biochem.* 56: 63-87

Hwang, C., Sinskey, A. J. and Lodish, H. F. (1992) Oxidized redox state of glutathione in the endoplasmic reticulum. *Science* 257: 1496-1502

Jackson, M. J., Nilson, T. and Peterson, P. A. (1990) Identification of a consensus motif for retention of transmembrane proteins in the endoplasmic reticulum. *EMBO J.* 9: 3153-3162.

Klausner, R. D. and Sitia, R. (1990) Protein degradation in the endoplasmic reticulum. *Cell* 62: 611-614

Klausner, R. D., Lippincott-Schwartz, J. and Bonifacino, J. S. (1990) The T-cell antigen receptor: Insights into organelle biology. *Ann. Rev. Cell. Biol.* 6: 403-433

Kunkel, T. A.(1985) Rapid and efficient site specific-mutagenesis without phenotype selection. *Proc. Natl. Acad. Sci. USA* 82: 488-492

Lankford, S. P, Cosson, P., Bonifacino, J. S. and Klausner, R. D. (1993) Transmembrane domain length affects charged-mediated retention and degradation of proteins within the endoplasmic reticulum. *J. Biol. Chem.* 268: 4814-4820

Leiden, J. M., Fraser, J. D. and Strominger, J. L. (1986) The complete structure of the T cell receptor genes from an alloreactive cytotoxic human T lymphocyte clone. *Immunogenetics* 24: 17-23

Lippincott-Schwartz, J., Bonifacino, J. S., Yuan, L. C. and Klausner, R. D. (1988) Degradation from the endoplasmic reticulum: disposing of newly synthesized proteins. *Cell* 54: 209-220

Lodish, H. F. (1988) Transport of secretory and membrane glycoprotein from the rough endoplasmic reticulum to the golgi. *J. Biol. Chem.* 263: 2107-2110

Manoloios, N., Bonifacino, J. S. and Klausner, R. D. (1990) Transmembrane helical interactions and the assembly of the T cell receptor complex. *Science* 249: 274-277

Munro, S. and Pelham, H. R. B. (1987) A C-terminal signal prevents secretion of lumenal ER proteins. *Cell* 49: 899-907

Pfeffer, S. R. and Rothman, J. E. (1987) Biosynthetic protein transport and sorting by the ER and Golgi. *Ann. Rev. Biochem.* 56: 829-852

Shin, J. K., Dunbrack, Jr. R. L., Lee, S. J. and Strominger, J. L.(1991) Signal for retention of transmembrane protein in the endoplasmic reticulum studied with CD4 truncation mutants.. *Proc. Natl. Acad. SCi. USA* 88: 1918-1922

Shin, J. K., Lee, S. J. and Strominger, J. L (1993) Translocation of TCR α chains into the lumen of the endoplasmic reticulum and their degradation. *Science* 259: 1901-1904

Sitia, R., Neuberger, M. S., Alberini, C., Bet, P., Fra, A., Valetti, C., Williams, G. and Milstein, C. (1990) Developmental regulation of IgM secretion: the role of carboxy-terminal cysteine. *Cell* 60: 781-790

Stafford, F. and Bonifacino, J. S. (1991) A permeabilized cell system identifies the endoplasmic reticulum as a site of protein degradation. *J. Cell. Biol.* 115: 1225-1236

Tiganos, E., Yao, X. J., Friborg, J., Daniel, N. and Cohen, E. A. (1997) Putative alpha-helical structures in the human immunodeficiency virus type 1 Vpu protein and CD4 are involved in binding and degradation of the CD4 molecule. *J. Virol.* 71: 4452-4460

Wikstorm, L. and Lodish, H. F. (1992) Endoplasmic reticulum degradation of a subunit of the asialoglycoprotein receptor in vitro. Vesicular transport from endoplasmic reticulum is unnecessary. *J. Biol. Chem.* 267: 5-8

Wileman, T., Carson, G. R., Concino, M., Ahmed, A. and Terhorst, C. (1990) The γ and ε subunits of the CD3 complex inhibit pre-golgi degradation of newly synthesized T cell antigen receptors. *J. Cell. Biol.* 10: 973-986

Wileman, T., Kane, L. P., Young, J., Carson, G. R. and Terhorst, C. (1993) Association between subunit ectodomains promote T cell antigen receptor assembly and protect against degradation in the ER. *J. Cell. Biol.* 122: 67-78

Williams, M. A. and Fukuda, M. (1990) Accumulation of membrane glyco-proteins in lysosomes requires a tyrosine residue at a particular position in the cytoplasmic tail. *J. Cell. Biol.* 11:955-966