

# Selective Degradation of T Cell Antigen Receptor Chains Retained in a Pre-Golgi Compartment

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**Abstract.** We have examined the fate of newly synthesized T cell antigen receptor (TCR) subunits in a T cell hybridoma deficient in expression of the clonotypic  $\beta$  chain. Synthesis and assembly of the remaining chains proceed normally but surface expression of TCR chains is undetectable in these cells. A variety of biochemical and morphological techniques has been used to show that the TCR chains in these cells fail to be transported to any of the Golgi cisternae. Instead,

they are retained in a pre-Golgi compartment which is either part of or closely related to the endoplasmic reticulum. The CD3- $\delta$  chain is degraded by a non-lysosomal process that is inhibited at temperatures at or below 27°C. By contrast, the remaining chains (CD3- $\epsilon$ , CD3- $\gamma$ , and  $\zeta$ ) are very stable over 7 h. We propose possible mechanisms that may explain the differential fate of TCR chains retained in a pre-Golgi compartment.

THE study of multicomponent protein complexes can address many questions concerning the assembly, intracellular transport, and eventual fate of individual protein subunits. In this respect, the T cell antigen receptor (TCR)<sup>1</sup> is an excellent model for examining how a cell stoichiometrically assembles a multimeric protein complex and ensures that only complete receptor molecules are expressed on the cell surface. Both the mature human and murine T cell antigen receptors function to recognize foreign antigens via a clonotypic disulfide-linked heterodimer composed of an acidic Ti- $\alpha$  and a basic Ti- $\beta$  chain (reviewed by Marrack and Kappler, 1986). Noncovalently associated with the Ti- $\alpha\beta$  dimer are a group of three structurally similar proteins ( $\delta$ ,  $\epsilon$ ,  $\gamma$ ) termed the CD3 complex and a disulfide-linked homodimer  $\zeta_2$  (Borst et al., 1983; Kanellopoulos et al., 1983; Samelson et al., 1985a; Oettgen et al., 1986). Several studies in both the human and murine systems have shown the importance of concomitant expression of both Ti- $\alpha$  and Ti- $\beta$  with the CD3 chains and  $\zeta_2$  for cell surface expression of the TCR. In T cell mutants lacking Ti- $\alpha$  (Schmitt-Verhulst et al., 1987; Saito et al., 1987a), Ti- $\beta$  (Weiss and Stobo, 1984; Sussman et al., 1988b), or  $\zeta$  (Sussman et al., 1988a), marked reduction in surface expression of the remaining TCR chains occurs. Transfecting the genes for Ti- $\alpha$  or Ti- $\beta$  into mutant cells deficient in the synthesis of these chains can restore surface expression of functional TCRs in these cells (Ohashi et al., 1985; Saito et al., 1987b). T cell precursors isolated from fetal thymus or leukemic cells

corresponding to the earliest stages of intrathymic T cell maturation transcribe mRNAs for the  $\beta$  and CD3 chains but not for the  $\alpha$  chain (Furley et al., 1986; Van Dongen et al., 1987; Samelson et al., 1985b; Raulet et al., 1985; Snodgrass et al., 1985). TCR chains other than the  $\alpha$  chain are synthesized in these cells but fail to be transported to the plasma membrane (Hannum et al., 1987; Furley et al., 1986; Farr et al., 1985). Subsequently,  $\alpha$  chains are expressed. This allows the formation of the Ti- $\alpha\beta$  heterodimer which, in turn, allows the full TCR to appear on the cell surface (Furley et al., 1986; Van Dongen et al., 1987). All of these observations suggest that delivery of TCR complexes to the cell surface requires the synthesis of all of the receptor chains.

Studies carried out in our laboratory with the murine T cell hybridoma line, 2B4, identified one possible mechanism by which T cells prevent the surface expression of incompletely assembled receptor complexes. These studies showed that TCR chains are not synthesized in stoichiometric amounts (Minami et al., 1987). Rather, five of the receptor chains (Ti- $\alpha$ , Ti- $\beta$ , CD3- $\delta$ , CD3- $\epsilon$ , CD3- $\gamma$ ) are synthesized in great excess (70–90%) over the amount that is eventually assembled on the cell surface. The  $\zeta$  chain is synthesized in limiting amounts and therefore may determine the number of seven-member receptors made. While complete receptors are transported to the cell surface, partial complexes are not. Instead, they are degraded intracellularly, mostly in lysosomes. Consistent with the biosynthetic studies in 2B4 cells are more recent observations made in variant T cells lacking expression of the  $\zeta$  chain. In such cells, partial, five-member complexes were found to be routed to lysosomes where they were rapidly degraded (Sussman et al., 1988a).

In the present study, we provide evidence for the existence of an additional mechanism that causes retention of abnor-

1. *Abbreviations used in this paper:* DOM, 1-deoxymanojirimycin; Endo D, endo- $\beta$ -N-acetylglucosaminidase D; Endo H, endo- $\beta$ -N-acetylglucosaminidase H; ER, endoplasmic reticulum; MME, methionine methyl ester; NEPHGE, nonequilibrium pH gradient electrophoresis; TCR, T cell antigen receptor; TRAP, T cell receptor-associated protein.

mal complexes in intracellular compartments. We have found that newly synthesized TCR chains in a T cell hybridoma variant that fails to synthesize any  $\beta$  chains are assembled into partial complexes in the endoplasmic reticulum (ER) but fail to be transported to the Golgi system. Surprisingly, the fate of the individual chains within the ER varied. Whereas CD3- $\epsilon$ , CD3- $\gamma$ , and  $\zeta$  all remained stably trapped within the ER, CD3- $\delta$  underwent rapid degradation. The degradation was not inhibited by several inhibitors of lysosomal function but was inhibited by lowering the temperature to 27°C. We postulate several mechanisms that may explain the differential fate of TCR chains retained in a pre-Golgi compartment.

## Materials and Methods

### Reagents

1-Deoxymanojirimycin (DOM) and endo- $\beta$ -*N*-acetylglucosaminidase H (Endo H), were purchased from Genzyme (Boston, MA). Endo- $\beta$ -*N*-acetylglucosaminidase D (Endo D) was supplied by Calbiochem-Behring Corp. (La Jolla, CA). Pharmacological agents were obtained from the following sources: ammonium chloride from Aldrich (Milwaukee, WI); methionine methyl ester, chloroquine, and monensin from Sigma Chemical Co. (St. Louis, MO); and leupeptin from Boehringer Mannheim Biochemicals (Indianapolis, IN).

### Cells

The murine T cell hybridoma 2B4 is a cell line generated by fusion of pigeon cytochrome c-primed lymph node cells to the BW5147 thymoma cell line (Hedrick et al., 1982). As a consequence, these hybridoma cells produce mRNAs for four clonotypic chains, 2B4- $\alpha$ , BW5147- $\alpha$ , 2B4- $\beta$ , and BW5147- $\beta$  (Sussman et al., 1988b). The subclone 2B4.11, derived by limiting dilution (Ashwell et al., 1987), was used in these studies. The  $\beta$ -deficient T cell line 21.2.2 was derived by repetitive subcloning of 2B4.11 cells (Sussman et al., 1988b) and was kindly provided by Dr. J. Ashwell (National Cancer Institute). Northern blot analyses have revealed that 21.2.2 cells fail to express mRNAs for 2B4- $\beta$ , BW5147- $\beta$ , and 2B4- $\alpha$ , but do express mRNA for BW5147- $\alpha$  (Sussman et al., 1988b). The Chinese hamster ovary (CHO) mutant cell line, LEC1 (Stanley et al., 1975), was obtained from the American Type Culture Collection (Rockville, MD). All cells were cultured in medium as described previously (Samelson and Schwartz, 1984).

### Antibodies

The following antibodies were used to precipitate the TCR chains: anti- $\epsilon$ , 145-2C11 (2C11), monoclonal hamster IgG that binds to the murine  $\epsilon$  chain (Leo et al., 1987); anti- $\delta$ , affinity-purified anti-peptide antisera raised in a goat immunized with a COOH-terminal peptide of the murine  $\delta$  chain (Samelson et al., 1986b); anti- $\gamma$ , No. 125, polyclonal antisera raised in a rabbit immunized with purified  $\gamma$  and  $\epsilon$  chains; anti- $\zeta$ , No. 124, polyclonal antisera raised in a rabbit immunized with purified murine  $\zeta$  chain. This anti- $\zeta$  serum causes dissociation of  $\zeta$  from the rest of the complex (Weissman et al., 1986). For immunoprecipitation, anti- $\epsilon$ , - $\gamma$ , and - $\zeta$  antibodies were bound to protein A-agarose (Bethesda Research Laboratories, Gaithersburg, MD). Anti- $\delta$  was covalently coupled to CNBr-activated Sepharose beads (Pharmacia Fine Chemicals, Uppsala, Sweden). To precipitate transferrin receptor, human transferrin (iron saturated; Miles Scientific, Naperville, IL) was covalently coupled to CNBr-activated Sepharose beads as above and was kindly supplied by Dr. J. Harford (National Institute of Child Health and Human Development). The monoclonal hamster antibody, 500A2, binds to the murine  $\epsilon$  chain and was a generous gift from Dr. J. Allison (University of California at Berkeley). Peroxidase-conjugated, affinity-purified, goat anti-hamster IgG was purchased from Kirkegaard & Perry Laboratories, Inc. (Gaithersburg, MD).

### Radioiodination, Metabolic Labeling, Immunoprecipitation, and Electrophoresis

Lactoperoxidase-catalyzed radioiodination and electrophoretic analysis of samples were performed as previously described (Samelson et al., 1985a,

1986a). Cells were metabolically labeled with [<sup>35</sup>S]methionine or <sup>3</sup>H-leucine as follows. [<sup>35</sup>S]methionine: Cells were preincubated for 10 min at 37°C in methionine-free RPMI 1640 medium (Biofluids Inc., Rockville, MD) containing 5% FBS and labeled for 5–30 min at  $1 \times 10^7$  cells/ml with 0.25 mCi/ml [<sup>35</sup>S]methionine (Tran-<sup>35</sup>S-label; ICN Radiochemicals, Irvine, CA). <sup>3</sup>H-leucine: Cells were preincubated for 10 min at 37°C in leucine-free RPMI 1640 (Biofluids Inc.) and labeled for 15 min at  $5 \times 10^7$  cells/ml with 5 mCi/ml <sup>3</sup>H-leucine (ICN Radiochemicals). When indicated, labeled cells were chased with complete medium at the specified temperatures. Solubilization and immunoprecipitation were performed as previously described (Samelson et al., 1985a) except anti- $\delta$  and anti- $\gamma$  immunoprecipitates were washed in 0.1% SDS and 0.2% SDS/0.2% sodium deoxycholate, respectively. To quantitate radioactivity incorporated into protein, band intensities on autoradiograms were determined by scanning densitometry using a Hoefer densitometer and computer analyzed using the Appligation II data analysis program (Dynamics Solutions, Pasadena, CA).

### Endoglycosidase Treatments

Digestion with Endo H was performed by incubating immunoprecipitated proteins for 16 h at 37°C with 5 mU Endo H in 50  $\mu$ l of 0.1 M sodium phosphate buffer, pH 6.1, containing 0.1% Triton X-100, 0.03% SDS, and 20 mM EDTA. Digestion with Endo D was similarly performed with 2.5 mU Endo D in 50  $\mu$ l of 0.02 M sodium phosphate buffer, pH 6.5 containing 0.1% Triton X-100, and 6 mM EDTA.

### Electron Microscopy

The ultrastructural localization of CD3- $\epsilon$  chains was studied in 2B4 and 21.2.2 cells by preembedding, immunoperoxidase electron microscopy using the 500A2 antibody. Cells were grown on glass coverslips, fixed in formaldehyde, permeabilized, incubated with 500A2 followed by peroxidase-conjugated, goat anti-hamster IgG, reacted with diaminobenzidine hydrochloride/H<sub>2</sub>O<sub>2</sub>, and prepared for electron microscopy as described by Yuan et al., 1987.

## Results

### *A $\beta$ -Deficient T Cell Hybridoma Variant Fails To Express T Cell Receptor on the Cell Surface*

The T cell antigen receptor is a macromolecular complex composed of at least seven transmembrane chains. The clonotypic  $\alpha$  and  $\beta$  glycoproteins exist as a disulfide-linked heterodimer and are noncovalently associated with a disulfide-linked homodimer,  $\zeta_2$ , and three other invariant chains termed the CD3 complex. The CD3 chains include CD3- $\epsilon$  and two glycoproteins, CD3- $\delta$  and CD3- $\gamma$ . Surface expression of TCR chains was studied in two murine T cell hybridomas, 2B4 and 21.2.2. 2B4 cells synthesize all seven chains of the TCR (Samelson et al., 1985a). 21.2.2 is a variant of 2B4 deficient in the expression of  $\beta$  chains and was derived by repetitive subcloning of 2B4 cells (Sussman et al., 1988b). To analyze the expression of TCR on the cell surface, intact 2B4 and 21.2.2 cells were subjected to lactoperoxidase-catalyzed surface radioiodination, cells were lysed in detergent, and the solubilized proteins were immunoprecipitated with an anti-CD3- $\epsilon$  antibody. Samples were analyzed by two-dimensional nonreducing/reducing diagonal gels. Fig. 1 A shows that the anti- $\epsilon$  antibody coprecipitates the entire TCR complex of 2B4 cells. In contrast,  $\beta$ -deficient 21.2.2 cells have a complete lack of any detectable TCR subunits on the cell surface when anti- $\epsilon$  antibodies (Fig. 1 B) are used for immunoprecipitation. These results can be confirmed by fluorescent cell sorter analysis using anti- $\epsilon$  antibodies (data not shown).

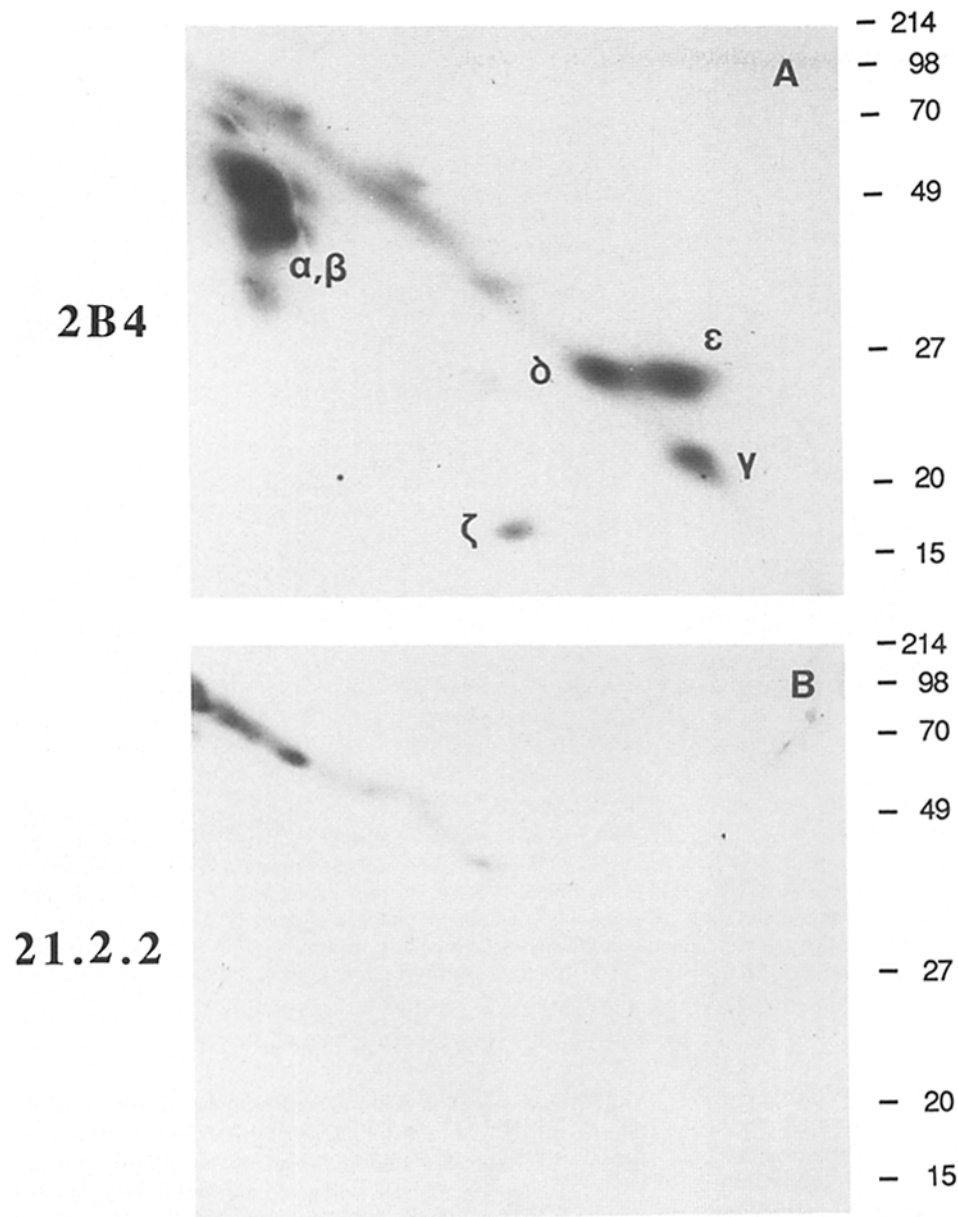


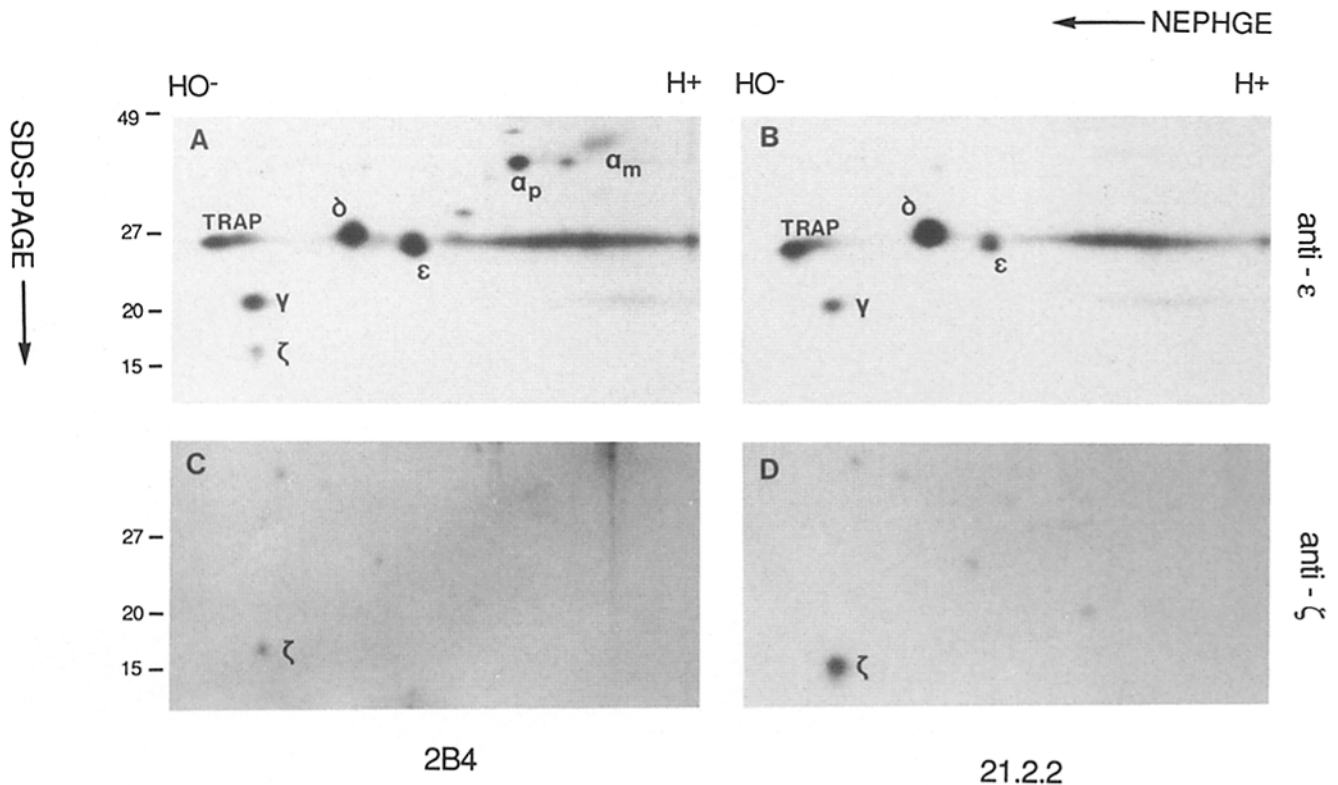
Figure 1. Surface expression of TCR chains in 2B4 and 21.2.2 T cell hybridomas. Intact cells were surface labeled by lactoperoxidase-catalyzed radioiodination. The cells were detergent solubilized and the lysates were immunoprecipitated with an anti- $\epsilon$  antibody. Immunoprecipitated proteins were analyzed by two-dimensional nonreducing/reducing diagonal SDS-PAGE on 13% gels. The positions of the TCR chains in 2B4 cells (A) or 21.2.2 cells (B) are indicated. The positions of  $M_r$  markers appear to the right of each panel; values are in  $10^{-3} \times M_r$ .

### Biosynthesis of TCR Chains Is Preserved in 21.2.2 Cells

To determine whether the  $\beta$ -deficient cells were able to synthesize the other TCR chains, we studied 21.2.2 and 2B4 cells by metabolic labeling with [ $^{35}$ S]methionine. Cells were pulse labeled, extracted with Triton X-100, and the solubilized proteins were immunoprecipitated with either an anti- $\epsilon$  antibody or an anti- $\zeta$  antibody. Samples were analyzed by two-dimensional nonequilibrium pH gradient electrophoresis (NEPHGE)/SDS-PAGE gels. Fig. 2 A shows the 2B4 control immunoprecipitation with anti- $\epsilon$  where all receptor subunits can be seen except  $\beta$  which labels poorly with [ $^{35}$ S]methionine. In intentionally overexposed autoradiograms, the  $\beta$  chain can be seen as a neutral 40-kD protein to the left of the  $\alpha_p$  protein. The precursor ( $\alpha_p$ ) and mature ( $\alpha_m$ ) forms of the  $\alpha$  subunit can be separated as shown due to a change in charge subsequent to the addition of sialic acid (Lippincott-Schwartz et al., 1988). The murine T cell recep-

tor-associated protein (TRAP), a protein that is transiently associated with the newly synthesized receptor chains, has recently been described by our laboratory (Bonifacino et al., 1988) and appears on this NEPHGE as two regions of radioactivity at 26 kD. TRAP that has completely entered the first dimension gel resolves as a very basic protein while the remainder migrates as a streak beginning at the origin of the NEPHGE tube.

Immunoprecipitation of TCR chains with anti- $\epsilon$  antibodies from 21.2.2 cells reveals only the CD3 chains ( $\delta$ ,  $\epsilon$ ,  $\gamma$ ) and the TRAP protein as shown in Fig. 2 B. The absence of coprecipitated  $\alpha$  and  $\zeta$  in 21.2.2 cells can represent either a failure to synthesize these chains or a failure to form a stable complex between  $\alpha$ ,  $\zeta$ , and the CD3 complex when  $\beta$  is not present. That these cells synthesize  $\zeta$  is confirmed by direct immunoprecipitation with anti- $\zeta$  antibodies (Fig. 2, C and D). Compared to 2B4 cells (Fig. 2 C), 21.2.2 seems to syn-



**Figure 2.** Biosynthesis of TCR chains in 2B4 and 21.2.2 cells. 2B4 cells (*A* and *C*) or 21.2.2 cells (*B* and *D*) were pulse-labeled with [<sup>35</sup>S]methionine for 30 min at 37°C before solubilization and immunoprecipitation with an anti-ε antibody (*A* and *B*) or an anti-ζ antibody (*C* and *D*). The immunoprecipitated proteins were analyzed by 13% NEPHGE/SDS-PAGE under reducing conditions. The identity of the TCR chains in these gels was established from previous work (Samelson et al., 1985*a*, 1986*a*). The positions of δ, ε, γ, and ζ are indicated. The position of TRAP which has completely entered the first-dimension gel is labeled. The remainder migrates as a streak beginning at the origin of the NEPHGE tube. α<sub>p</sub> and α<sub>m</sub> indicate the precursor and mature forms of the α chains, respectively. The β chains are poorly labeled under these conditions and are not apparent in *A*. The positions of *M<sub>r</sub>* markers are shown to the left of the figure. Values are expressed as 10<sup>-3</sup> × *M<sub>r</sub>*.

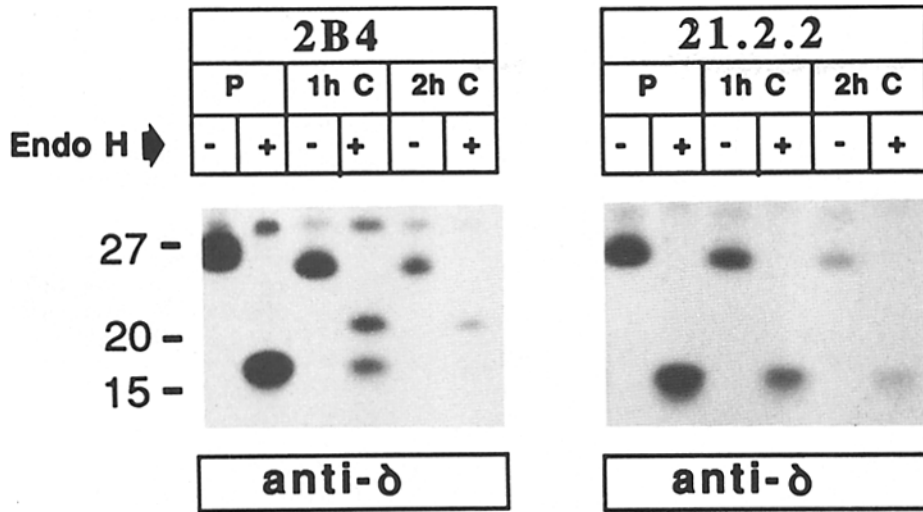
thesize an even greater amount of ζ (*D*). The biosynthesis of the BW5147-α chain in 21.2.2 cannot be studied directly due to the lack of an appropriate antibody. We cannot detect any coprecipitated BW5147-α with the CD3 chains when anti-ε antibodies are used (Fig. 2 *B*) but we cannot rule out the possibility that the α chain is not synthesized in these cells although the mRNA is expressed. Our observation is in contrast to the recent detection of α-CD3 complexes in human T cell lines (Alarcon et al., 1988; Koning et al., 1988). From these pulse-labeling studies of 21.2.2, we conclude that biosynthesis of the CD3 subunits continues in the absence of β subunit expression and that the lack of surface receptor expression is not due to impaired biosynthesis of the remaining chains.

#### **Receptor Subunits Are Not Transported to the Medial Golgi System in 21.2.2 Cells**

Further studies were conducted to trace the route followed by the receptor chains of 21.2.2 cells after biosynthesis in the endoplasmic reticulum. Since these chains failed to reach their ultimate destination on the plasma membrane, we can contrast the intracellular membrane transport of CD3 components in 21.2.2 with that of 2B4. The transport of glycosylated receptor subunits through the Golgi system can be monitored by following acquisition of resistance to diges-

tion by Endo H. This enzyme catalyzes the hydrolysis of the chitobiose core of high mannose oligosaccharides that exist before reaching the medial Golgi system (Tarentino and Maley, 1974). Once glycoproteins arrive at the medial Golgi, *N*-acetylglucosamine is added and the carbohydrate intermediates that are generated are no longer sensitive to cleavage by Endo H (Dunphy et al., 1985; reviewed by Kornfeld and Kornfeld, 1985). We analyzed processing of glycosylated CD3 chains in 2B4 and 21.2.2 cells by pulse labeling cells with [<sup>35</sup>S]methionine and chasing for 1 or 2 h at 37°C. TCR subunits were isolated with anti-δ antibodies and anti-γ antibodies. Half of each immunoprecipitate was treated with Endo H, and anti-δ samples were analyzed under nonreducing SDS-PAGE gels (Fig. 3). Anti-γ samples for 21.2.2 and 2B4 were analyzed under reducing SDS-PAGE gels and two-dimensional NEPHGE gels, respectively (data not shown). The Endo H-resistant species of CD3-δ and -γ in 2B4 and 21.2.2 cells were quantitated by scanning densitometry of autoradiograms and shown in Table I.

The murine CD3-δ chain contains three *N*-linked carbohydrate chains, one of which is processed to complex carbohydrates in the mature δ chain while two remain in a high mannose form (van den Elsen et al., 1985; Chen, C., unpublished observations). The single *N*-linked carbohydrate chain of the murine CD3-γ chain is processed to complex



**Figure 3.** Medial Golgi processing of CD3- $\delta$  occurs in 2B4 cells but not in 21.2.2 cells. 2B4 and 21.2.2 cells were pulse-labeled for 15 min at 37°C with [<sup>35</sup>S]methionine and chased for 1 or 2 h in complete medium at 37°C. Cells were detergent solubilized and the  $\delta$  chain was immunoprecipitated with anti- $\delta$  antibodies. Half of the immunoprecipitated proteins were treated with Endo H and samples were analyzed by 13% SDS-PAGE gels under nonreducing conditions. The positions of  $M_r$  markers are indicated to the left of the figure; values are in  $10^{-3} \times M_r$ . P, pulse; 1h C, 1 hour chase; 2h C, 2 hour chase.

sugars in the mature glycoprotein (Krissansen et al., 1987; Haser et al., 1987; Chen, C., unpublished observations). Fig. 3 shows that after a 15-min pulse, all newly synthesized 26–28-kD  $\delta$  precursors are sensitive to cleavage by Endo H to a 16-kD species in both cell lines. After a 1-h chase, 55% of the  $\delta$  population in 2B4 cells becomes partially resistant to Endo H as evidenced by the appearance of a 21-kD species (Fig. 3 and Table I). At 2 h, 75% of the remaining  $\delta$  is partially Endo H resistant in 2B4 cells. In contrast, no detectable Endo H-resistant forms of  $\delta$  are seen in 21.2.2 cells over a 2-h (or longer) chase period. The 2B4  $\delta$  precursor forms appear as a nondiscrete band around 26–28-kD, whereas the  $\delta$  precursor in 21.2.2 is a discrete 26-kD species. Despite this, migration of the deglycosylated newly synthesized  $\delta$  is identical in the two cell lines. These variable  $\delta$  precursor forms may reflect different rates of carbohydrate processing in the rough endoplasmic reticulum of 2B4 and 21.2.2 cells. Table I shows that while 66% of CD3- $\gamma$  in 2B4 cells acquires Endo H resistance over a 2-h chase period, CD3- $\gamma$  in 21.2.2 does not. These findings imply that in 21.2.2 cells,  $\delta$ ,  $\gamma$ , and the associated CD3- $\epsilon$  chain do not reach the medial Golgi

but are blocked in a premedial Golgi compartment. The data in Table I also suggest that transport of TCR chains in these murine T cells is not a slow process as has recently been shown by Alarcon et al. (1988) in human T cells. We find that >50% of CD3- $\delta$  chains are processed in the medial Golgi of 2B4 cells after a 1-h chase.

Of note in Fig. 3 is the relative rapid loss of newly synthesized  $\delta$  chains in both 2B4 and 21.2.2 cells. The disappearance seen in 2B4 cells over a 2-h chase period can be attributed mostly to lysosomal degradation of excess  $\delta$  after passage through the Golgi system (Minami et al., 1987). Surprisingly, a similar rate of  $\delta$  degradation is seen in 21.2.2 cells. We analyze this degradation of  $\delta$  in more detail in later figures.

#### Receptor Subunits Are Blocked in a Pre-cis-Golgi Compartment in 21.2.2 Cells

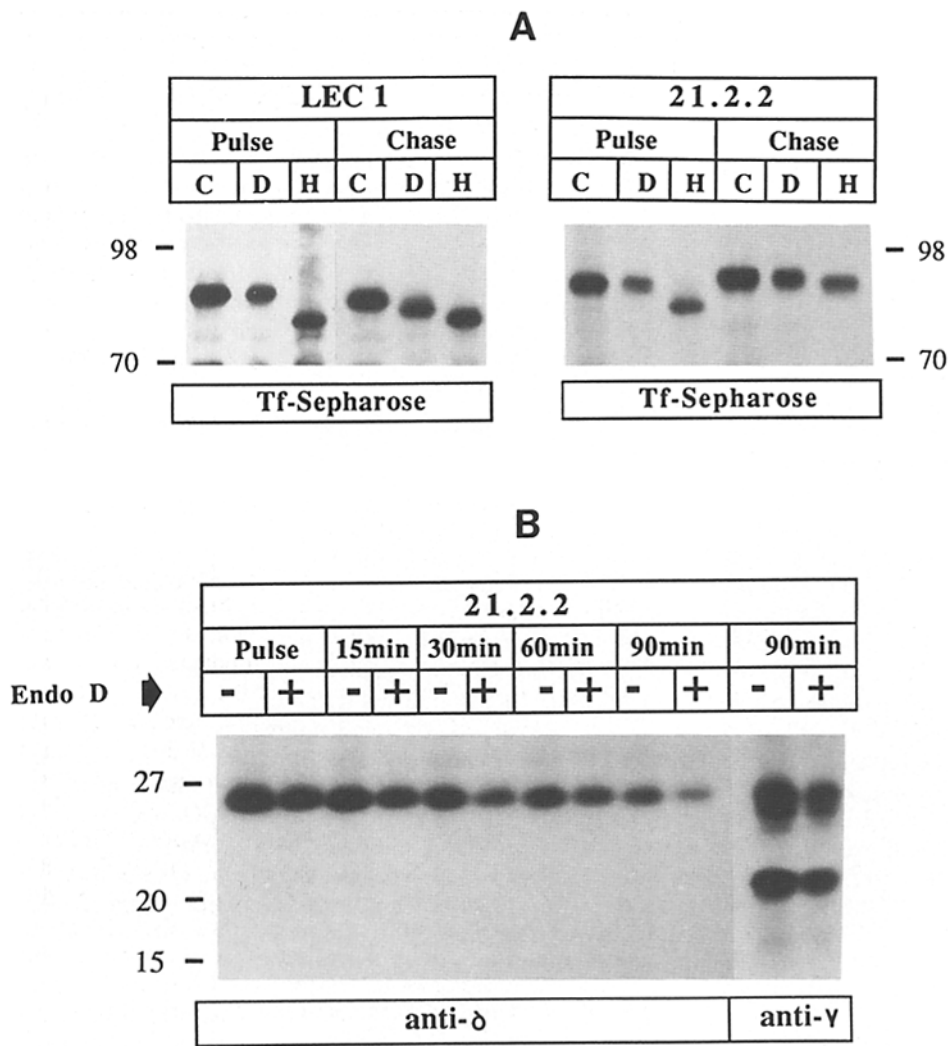
To determine whether the CD3- $\delta$  and - $\gamma$  chains in 21.2.2 cells accumulate in the *cis*-Golgi stacks, we analyzed the sensitivity of these chains to cleavage by Endo D. Endo D hydrolyzes the di-*N*-acetylchitobiosyl linkage of asparagine-linked oligosaccharides only when the  $\alpha$ -mannose residue that is in an  $\alpha$ -1,3 linkage to the trisaccharide core is unsubstituted in the 2 position (Koide and Muramatsu, 1974). Only glycoproteins that have been processed by the *cis*-Golgi-associated  $\alpha$ -1,2 mannosidase I to  $\text{Man}_5\text{GlcNAc}_2$  oligosaccharide intermediates are Endo D sensitive.

Because we were unable to accumulate the transiently present but obligatory  $\text{Man}_5\text{GlcNAc}_2$  intermediate of CD3- $\delta$  in 2B4 cells as a positive control, we examined the processing of the transferrin receptor in LEC1 cells as our control. LEC1 cells are CHO fibroblast cells that are missing the enzyme *N*-acetylglucosaminyltransferase I (Stanley et al., 1975). Consequently, all  $\text{Man}_5\text{GlcNAc}_2$  species in this cell line do not undergo processing to complex oligosaccharide forms containing *N*-acetylglucosamine, galactose, and sialic acid, and should remain Endo D sensitive. Each chain of the transferrin receptor, which is a disulfide-linked homodimer comprised of two 90-kD glycoproteins in the hamster and two 95-kD glycoproteins in the mouse (Van Agthoven et al.,

**Table I.** Quantitation of Processed CD3- $\delta$  and - $\gamma$  Chains to Endo H-resistant Forms in 2B4 and 21.2.2 Cells

Chase time (in hours)	Endo H-resistant (in percent)			
	CD3- $\delta$		CD3- $\gamma$	
	2B4	21.2.2	2B4	21.2.2
0	2	0	0	0
1	55	0	ND	0
2	75	0	66	0

2B4 and 21.2.2 cells were pulse labeled for 15 min with [<sup>35</sup>S]methionine and chased in complete medium for 1 or 2 h at 37°C. CD3- $\delta$  samples were prepared as described in the legend of Fig. 3. CD3- $\gamma$  chains were immunoprecipitated with anti- $\gamma$  antibodies and half of the immunoprecipitated proteins was treated with Endo H. Anti- $\gamma$  samples from 21.2.2 were analyzed by 13% SDS-PAGE gels under reducing conditions. 2B4 anti- $\gamma$  samples were resolved by NEPHGE/SDS-PAGE under reducing conditions. Quantitation of Endo H-resistant species was performed by scanning densitometry of bands on the autoradiograms. The percent of remaining chains which were Endo H resistant are shown for each time point.



**Figure 4.** CD3- $\delta$  and - $\gamma$  chains in 21.2.2 cells do not accumulate in the *cis*-Golgi. (A) LEC1 CHO fibroblast cells and 21.2.2 cells were pulse labeled for 5 min at 37°C with [<sup>35</sup>S]methionine and chased for 90 min in complete medium at 37°C. Transferrin receptor was precipitated from detergent-solubilized cell lysates with human transferrin coupled to Sepharose. Samples were divided into thirds and were either untreated (lanes designated C for control), treated with Endo D (lanes designated D), or treated with Endo H (lanes designated H) before analysis by 10% SDS-PAGE gels under reducing conditions. The positions of *M<sub>r</sub>* markers are indicated. (B) 21.2.2 cells were pulse labeled for 10 min at 37°C with [<sup>35</sup>S]methionine and chased at 37°C in complete medium for varying times as indicated. CD3- $\delta$  and - $\gamma$  chains were immunoprecipitated from detergent-solubilized cell lysates with anti- $\delta$  and anti- $\gamma$  antibodies, respectively. Half of each sample was treated with Endo D and analyzed by 13% SDS-PAGE gels under non-reducing and reducing conditions for anti- $\delta$  and anti- $\gamma$  samples, respectively. The anti- $\gamma$  antibody immunoprecipitates the  $\delta$  chain in addition to the  $\gamma$  chain after washing with 0.2% SDS/0.2% deoxycholic acid. The positions of *M<sub>r</sub>* markers are indicated. Values are expressed as  $10^{-3} \times M_r$ .

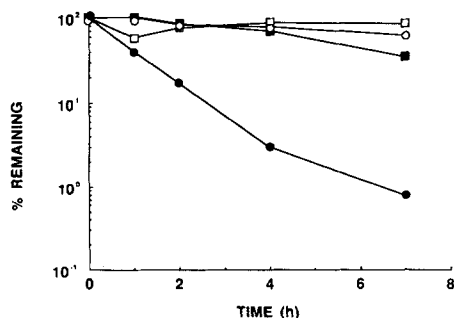
1984; Testa, 1985), has three N-linked carbohydrate chains. Two of these chains remain in a high mannose form in the mature receptor while one is processed to complex carbohydrates (Newman et al., 1982). Fig. 4 A shows that in LEC1 cells, the transferrin receptor is resistant to Endo D in a 5-min pulse but becomes partially sensitive to Endo D after a 90-min chase. The protein remains Endo H sensitive at both time points, consistent with its failure to be processed to complex carbohydrates in these cells. In 21.2.2 cells, the mouse transferrin receptor remains Endo D resistant in both the pulse and chase time points. Partial Endo H resistance is acquired after a 90-min chase. Fig. 4 B shows that the CD3- $\delta$  and - $\gamma$  chains in 21.2.2 never become Endo D sensitive at any time points examined. These studies allow us to draw the following conclusions. (a) The TCR CD3- $\delta$  and - $\gamma$  chains fail to acquire Endo D sensitivity and therefore do not accumulate in the *cis*-Golgi. This implies that these chains, and presumably all the TCR chains of 21.2.2, most likely are retained in a pre-Golgi compartment which may or may not be the ER. (b) The Golgi system of 21.2.2 cells is intact and functional since other glycoproteins, such as the transferrin receptor, appear to be transported through the Golgi stacks

where Endo H resistance is acquired in the medial Golgi system.

We sought to further define the carbohydrate content of N-linked sugars on CD3- $\delta$  in 21.2.2 cells by using the carbohydrate processing inhibitor 1-deoxymannojirimycin (DOM). DOM inhibits the ER mannosidase I and allows Man<sub>8-9</sub>GlcNAc<sub>2</sub> species to accumulate in cells. When 21.2.2 cells were preincubated in the presence of DOM for 2 h, pulsed for 5 min, and chased for 1 h in the presence of DOM, the Man<sub>8-9</sub>GlcNAc<sub>2</sub> species of CD3- $\delta$  was clearly seen as a 27-kD glycoprotein. In the absence of DOM,  $\delta$  was able to mature to a slightly smaller glycoprotein of 26 kD which remained sensitive to Endo H but resistant to Endo D. From these studies, we conclude that the final species of  $\delta$  in 21.2.2 contains between 6 and 8 mannose residues, consistent with its localization in a pre-Golgi compartment.

#### Differential Fate of TCR Chains in 21.2.2 Cells

From data shown in Fig. 3, loss of CD3- $\delta$  was seen over a 2-h chase period in 21.2.2 cells. This observed loss of immunoprecipitable  $\delta$  is not due to secretion into the medium



**Figure 5.** Differential fate of TCR chains in 21.2.2 cells. 21.2.2 cells were pulse labeled for 15 min at 37°C with [<sup>35</sup>S]methionine and chased for 1, 2, 4, or 7 h at 37°C. Labeled proteins were extracted in detergent and TCR chains were immunoprecipitated with anti- $\delta$ , anti- $\gamma$ , anti- $\epsilon$ , and anti- $\zeta$  antibodies. Anti- $\delta$  and anti- $\gamma$  samples were analyzed by 13% SDS-PAGE gels under reducing conditions. Anti- $\epsilon$  and anti- $\zeta$  samples were resolved by 13% two-dimensional nonreducing/reducing diagonal gels. Receptor chains were quantitated by scanning densitometry of autoradiograms. □,  $\gamma$ ; ○,  $\epsilon$ ; ■,  $\zeta$ ; ●,  $\delta$ .

nor to failure to solubilize (i.e., no additional  $\delta$  was recovered when cells were extracted in the presence of SDS). To ensure that there was no conformational change resulting in the apparent loss of  $\delta$  due to loss of recognition of the anti- $\delta$  antibody, we performed pulse-chase studies using cycloheximide to arrest new  $\delta$  synthesis. At different times after the addition of cycloheximide, whole cell lysates were resolved by SDS-PAGE and  $\delta$  was quantitated by immunoblot analysis. This experiment demonstrated the identical kinetics for  $\delta$  loss seen by metabolic pulse-chase experiments (data not shown). What then is the fate of the other CD3 chains of 21.2.2 in this pre-Golgi compartment? To address this question, we pulsed 21.2.2 cells with [<sup>35</sup>S]methionine and chased for various times over 7 h at 37°C. TCR subunits were isolated with anti- $\delta$ , anti- $\gamma$ , anti- $\epsilon$ , and anti- $\zeta$  antibodies. Anti- $\delta$  and anti- $\gamma$  samples were analyzed under reducing SDS-PAGE gels. Anti- $\epsilon$  and anti- $\zeta$  samples were run on two-dimensional nonreducing/reducing diagonal gels. Receptor chains were quantitated by scanning densitometry of autoradiograms and plotted in Fig. 5. CD3- $\delta$  has a half-life of 45–60 min in the pre-Golgi compartment as shown in Fig. 5. In striking contrast to the fate of  $\delta$ , the other TCR chains,  $\gamma$ ,  $\epsilon$ , and  $\zeta$ , remain extremely stable with half-lives of several hours. We detect similar kinetics of  $\delta$  degradation and  $\gamma$  stability over a 7-h chase period when these chains are coprecipitated with anti- $\epsilon$  antibodies. Thus, the fates of  $\delta$  and  $\gamma$  are not dependent on the antibodies used for immunoprecipitation. In addition, when 21.2.2 cells were treated with tunicamycin or castanospermine, the resultant lack of proper carbohydrate chains on  $\delta$  and  $\gamma$  did not alter the fates of either chain in these cells.

To address the question of whether  $\delta$  loss occurs in the same compartment of the ER as the site of synthesis and assembly of receptor subunits, we examined the fate of  $\epsilon$  chains in 21.2.2 cells when coprecipitated with anti- $\delta$  antibodies. If  $\delta$  degradation occurs in the same compartment as the site of assembly, we predict that  $\epsilon$  could continue to be coprecipitated by anti- $\delta$  antibodies, even at chase time points when previously synthesized  $\delta$  is undergoing degradation. This prediction assumes that the resulting  $\epsilon$ - $\gamma$  complex can rebind newly synthesized  $\delta$  chains that are not labeled during

the chase. If  $\delta$  degradation and receptor assembly occur in separate compartments within the ER, we would expect  $\delta$  and  $\epsilon$  to be lost with similar kinetics when immunoprecipitated with anti- $\delta$  antibodies because the resulting  $\epsilon$ - $\gamma$  complex would have no access to newly synthesized  $\delta$  chains. To test this hypothesis, 21.2.2 cells were pulsed with [<sup>35</sup>S]methionine and chased for various times over 7 h at 37°C. TCR subunits were isolated with anti- $\delta$  antibodies and immunoprecipitates were washed in the absence of 0.1% SDS to prevent dissociation of assembled CD3 complexes. The samples were run on two-dimensional nonreducing/reducing diagonal gels followed by quantitation of receptor chains by scanning densitometry of autoradiograms (data not shown). Pulse-labeled  $\epsilon$  as well as  $\delta$  chains were lost over time with half-lives of 45–60 min. These results indicate that after the loss of newly synthesized  $\delta$  from  $\delta$ - $\epsilon$ - $\gamma$  complexes, the surviving chains cannot reassemble with subsequently synthesized  $\delta$  chains.

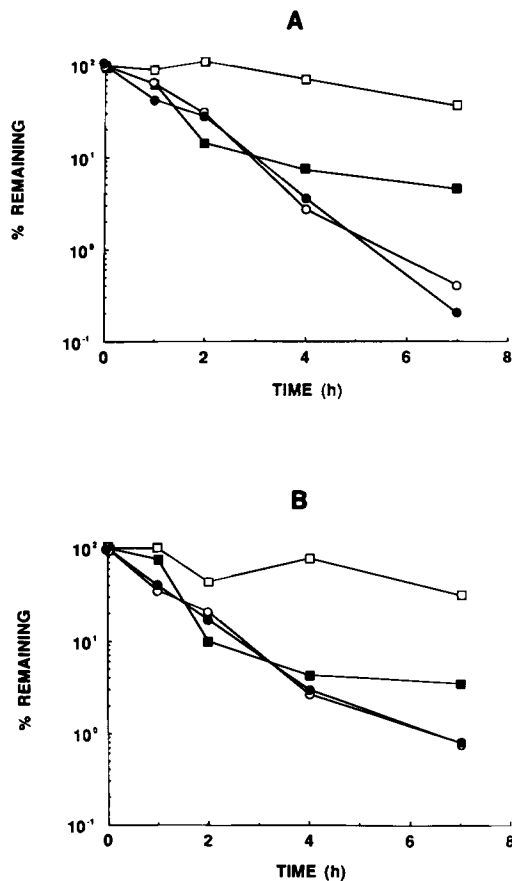
### **CD3- $\delta$ Degradation in 21.2.2 Is Not Inhibited by a Variety of Lysosomal Inhibitors**

Having determined that only the CD3- $\delta$  chain in 21.2.2 cells undergoes rapid degradation, we sought to determine to what extent, if at all, the pre-Golgi degradation of  $\delta$  in these cells involved lysosomes. To test this, we conducted pulse-chase studies of  $\delta$  in 21.2.2 and 2B4 cells in the absence and presence of several inhibitors of lysosomal function: 50 mM NH<sub>4</sub>Cl, 20 mM methionine methyl ester (MME), 100  $\mu$ M chloroquine, 100  $\mu$ g/ml leupeptin, and 7  $\mu$ g/ml monensin (Wibo and Poole, 1974; Livesey et al., 1980; Seglen, 1983; Rote and Rechsteiner, 1983; Ohkuma and Poole, 1981; Poole and Ohkuma, 1981; Reeves et al., 1981; Tartakoff, 1983; Libby and Goldberg, 1978). Fig. 6 clearly shows the differential effects of NH<sub>4</sub>Cl and MME on  $\delta$  degradation in 21.2.2 and 2B4 cells. NH<sub>4</sub>Cl is effective in completely inhibiting  $\delta$  degradation in 2B4 cells over 2 h while it has no effect on  $\delta$  degradation in 21.2.2 cells (Fig. 6A). At 7 h, the inhibition of lysosomal degradation by NH<sub>4</sub>Cl in 2B4 cells is not complete but is still significant (40 and 4% of  $\delta$  remain in the presence and absence of NH<sub>4</sub>Cl, respectively). MME is equally effective in disrupting lysosomal function in 2B4 cells but has no significant effect on  $\delta$  degradation in 21.2.2 cells (Fig. 6B). Fig. 7 shows that other lysosomal inhibitors, including chloroquine, monensin, and leupeptin, similarly fail to retard the degradation of  $\delta$  in 21.2.2 cells. Thus, the data suggest that CD3- $\delta$  is indeed undergoing degradation by two distinct and different degradative systems in 2B4 and 21.2.2 cells. CD3- $\delta$  is largely degraded by a lysosomal pathway in 2B4 cells but by a nonlysosomal system in 21.2.2 cells. From data described in this paper, we propose that this novel degradative system in T cells is localized to a pre-Golgi compartment.

### **Effect of Temperature on Pre-Golgi Degradation of CD3- $\delta$ in 21.2.2**

Many biochemical and cellular processes have been shown to exhibit characteristic temperature dependence curves. With regard to intracellular protein degradation, temperature can affect several events in this process, some of which can be rate limiting. These include local protein unfolding (Pace, 1975; Snoke and Neurath, 1950) or other structural changes,

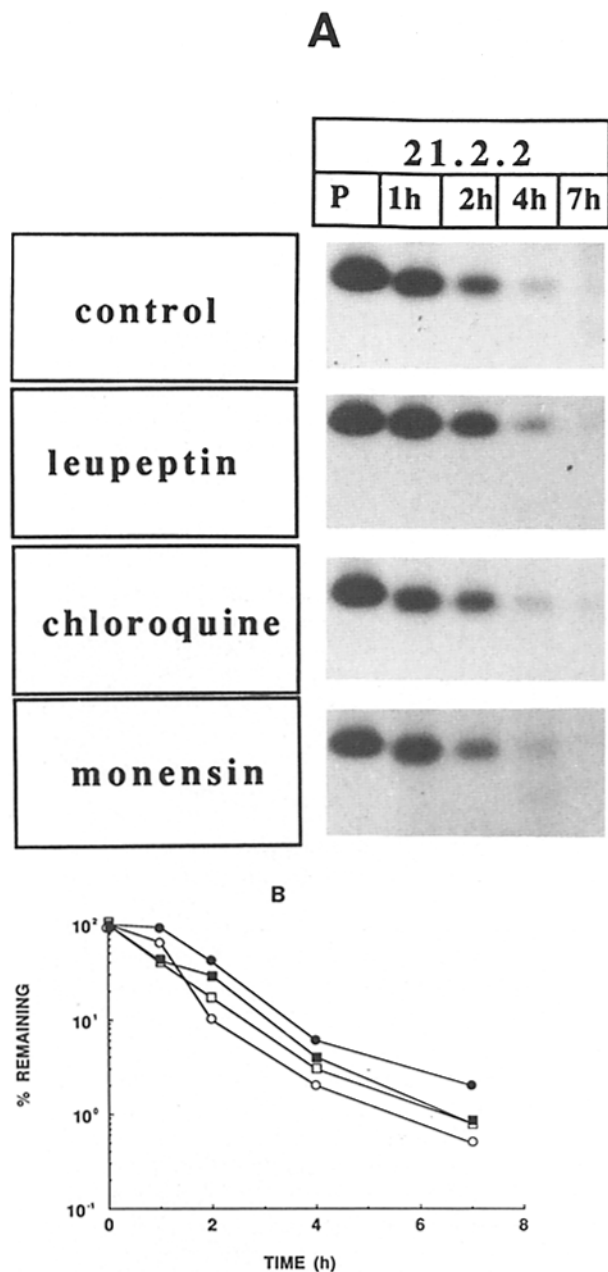




**Figure 6.** Fate of CD3- $\delta$  in 2B4 and 21.2.2 cells: differential effect of NH<sub>4</sub>Cl and MME. (A) 2B4 and 21.2.2 cells were preincubated with no drug (control) or with NH<sub>4</sub>Cl (50 mM) for 1 h at 37°C before pulse labeling with [<sup>35</sup>S]methionine for 15 min at 37°C. Cells were chased in complete medium in the absence (control) or presence of 50 mM NH<sub>4</sub>Cl for 1, 2, 4, or 7 h at 37°C. CD3- $\delta$  was immunoprecipitated from cell lysates with anti- $\delta$  antibodies and samples were analyzed by 13% SDS-PAGE gels under nonreducing conditions. Receptor chains were quantitated by scanning densitometry of autoradiograms. (B) 2B4 and 21.2.2 cells were preincubated with no drug (control) or with MME (20 mM) for 2 h at 37°C before pulse labeling with <sup>3</sup>H-leucine for 15 min at 37°C. Cells were chased in complete medium in the absence (control) or presence of drug for 1, 2, 4, or 7 h at 37°C. CD3- $\delta$  was isolated and analyzed as described in A. (A) □, 2B4 NH<sub>4</sub>Cl; ■, 2B4 control; ○, 21.2.2 NH<sub>4</sub>Cl; ●, 21.2.2 control. (B) □, 2B4 MME; ■, 2B4 control; ○, 21.2.2 MME; ●, 21.2.2 control.

proteolytic cleavage by specific enzymes (Yang et al., 1977), and membrane transport of proteins to the degradative organelle (Brahm, 1977). Saraste et al. (1986) have shown that the movement of newly synthesized proteins out of the ER is blocked by incubating cells at 16°C. In 2B4 cells, we have seen that maturation of TCR chains and their subsequent delivery to lysosomes are inhibited when cells are placed at 16°C (Bonifacino et al., 1988).

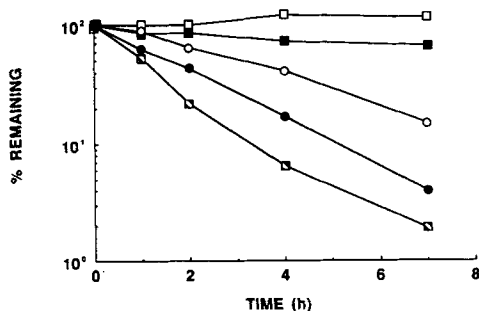
To test the temperature dependence of pre-Golgi CD3- $\delta$  degradation in 21.2.2 cells, we pulsed cells with [<sup>35</sup>S]methionine and chased for various times over 7 h at five different temperatures: 37, 34, 30, 27, and 20°C. Cells were lysed,  $\delta$  subunits were isolated with anti- $\delta$  antibodies, and the sam-



**Figure 7.** Lysosomal inhibitors do not affect the fate of CD3- $\delta$  chains in 21.2.2 cells. (A) 21.2.2 cells were preincubated with no drug (control) or with the following lysosomal inhibitors at 37°C for 1 h: leupeptin (100  $\mu$ g/ml), chloroquine (100  $\mu$ M), and monensin (7  $\mu$ g/ml). Cells were pulse labeled for 15 min at 37°C with [<sup>35</sup>S]methionine and chased in complete medium in the absence (control) or presence of drug for 1, 2, 4, or 7 h at 37°C. CD3- $\delta$  was isolated and analyzed as described in the legend to Fig. 6 A. (B) Scanning densitometry of the autoradiograms shown in A. ●, leupeptin; ■, chloroquine; □, control; ○, monensin.

ples were run on nonreducing SDS-PAGE gels. The CD3- $\delta$  chain was quantitated by scanning densitometry of autoradiograms and plotted in Fig. 8. The 37°C control curve shows the exponential degradation of  $\delta$  with a half-life of 60 min, consistent with previous experiments. Lowering the temperature by 3°C to 34°C increased the half-life of  $\delta$  to 100 min.





**Figure 8.** Effect of temperature on nonlysosomal CD3- $\delta$  degradation in 21.2.2 cells. 21.2.2 cells were pulse labeled for 15 min at 37°C with [<sup>35</sup>S]methionine and chased for 1, 2, 4, or 7 h at the indicated temperatures. CD3- $\delta$  was isolated and analyzed as described in the legend to Fig. 6 A. □, 20°C; ■, 27°C; ○, 30°C; ●, 34°C; ■, 37°C.

Cells incubated at 30°C exhibited dramatic stabilization of  $\delta$  to degradation (half-life = 190 min) while at temperatures of 27°C or lower, the degradation of  $\delta$  was completely inhibited. Thus, the nonlysosomal, pre-Golgi degradation observed in 21.2.2 cells is completely inhibited at temperatures that do not block ER-Golgi transport. An explanation for the extreme temperature sensitivity of pre-Golgi degradation in these cells requires further characterization.

#### **Localization of CD3- $\epsilon$ in 2B4 and 21.2.2 Cells by Electron Microscopy**

The ultrastructural distribution of CD3- $\epsilon$  chains was examined in 2B4 and 21.2.2 cells by immunoperoxidase electron microscopy (Fig. 9). We chose to examine the steady-state levels of CD3- $\epsilon$  rather than CD3- $\delta$  because of the stability of intracellular  $\epsilon$  in 21.2.2 cells and the availability of a useful anti- $\epsilon$  antibody. Cells were permeabilized with saponin and incubated with 500A2, an anti- $\epsilon$  monoclonal antibody. Fig. 9 A shows HRP staining of 2B4 cells in several structures including ER cisternae, nuclear envelope, Golgi cisternae, lysosomes, and plasma membrane. No staining of mitochondria and cytoplasm is observed. The morphological data are consistent with the biochemical evidence for movement of TCR complexes containing CD3- $\epsilon$  out of the ER to the Golgi complex in 2B4 cells. Incomplete complexes containing CD3- $\epsilon$  are delivered to lysosomes, which are clearly stained in Fig. 9 A, while complete TCR complexes are transported to the cell surface. In contrast, Fig. 9 B shows predominant HRP staining of ER cisternae, nuclear envelope, and transitional elements and vesicles in 21.2.2 cells. Little or no staining of the Golgi cisternae, lysosomes, plasma membrane, mitochondria, or cytoplasm is detected. The data are consistent with our biochemical data suggesting retention of TCR chains, including CD3- $\epsilon$ , in a pre-Golgi compartment in 21.2.2 cells.

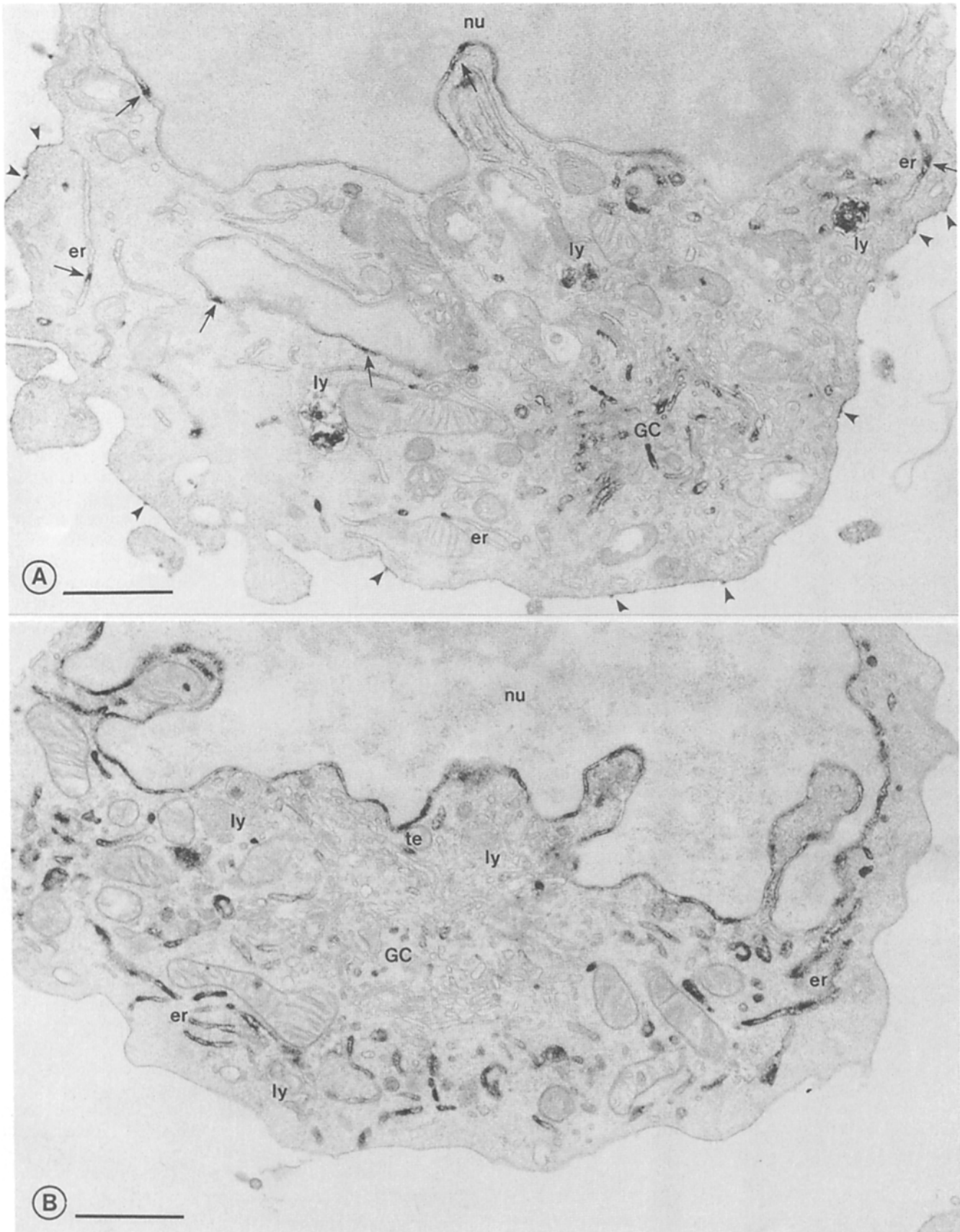
#### **Discussion**

Recent work on a variety of membrane glycoproteins reveals the highly selective steps of transport of newly synthesized proteins from the rough ER to the Golgi apparatus to the plasma membrane (reviewed by Lodish, 1988). Taken to-

gether, the data point to the importance of attaining proper conformations before proteins can mature from the site of synthesis in the ER to the Golgi. While in the ER, most integral membrane proteins undergo maturation and modification such as folding, glycosylation, disulfide bond formation, and oligomerization. One well-characterized viral glycoprotein, the influenza hemagglutinin (HA) glycoprotein spike, forms a trimer of three identical HA<sub>0</sub> polypeptides in the ER before the protein is transported to the Golgi (Gething et al., 1986; Copeland et al., 1986). Mutants of the HA polypeptide have been isolated that fail to be transported out of the rough ER (Gething et al., 1986). They have been shown to be blocked at different stages of the folding process (Gething et al., 1986; Doyle et al., 1985). The wild-type vesicular stomatitis virus (VSV) G glycoprotein also forms an oligomer while it is in the ER (Kreis and Lodish, 1986; Doms et al., 1987). Studies of a temperature-sensitive mutation in the gene encoding this protein (ts045) have shown that at the nonpermissive temperature, 39°C, the G protein remains as a monomer and is retained in the rough ER. At the lower permissive temperature, 32°C, oligomerization of the G protein occurs and allows normal movement of the protein through the Golgi to the cell surface (Kreis and Lodish, 1986). The human major histocompatibility antigens, HLA-A and -B, are oligomeric transmembrane glycoproteins each composed of a polymorphic heavy chain associated with a water-soluble nonglycosylated polypeptide,  $\beta_2$ -microglobulin (Ploegh et al., 1979; Owen et al., 1980). Association of the  $\beta_2$ -microglobulin with the heavy chain has been shown to be necessary for subsequent processing and intracellular transport of the heavy chain after synthesis in the ER (Ploegh et al., 1979; Owen et al., 1980; Sege et al., 1981). In addition, proper folding of the heavy chain itself is required for transport of the murine H-2K<sup>b</sup> molecule to the cell surface (Williams et al., 1988). Finally, retention in the ER has been described for both secreted and transmembrane forms of immunoglobulin heavy chains when light chains are not synthesized (Bole et al., 1986). Recently, Sitia et al. (1987) have also described retention of  $\mu_m$  heavy chains in transfected plasmacytomas that do not synthesize  $\mu_s$ .

We have characterized the assembly and intracellular transport of TCR chains in a T cell hybridoma lacking the expression of the  $\beta$  subunit. In normal 2B4 T cell hybridomas expressing the complete seven-member TCR on the cell surface, five of the chains ( $\alpha$ ,  $\beta$ ,  $\delta$ ,  $\epsilon$ ,  $\gamma$ ) are synthesized in great excess over the amount that eventually assembles into mature complexes (Minami et al., 1987; Lippincott-Schwartz et al., 1988; Chen, C., unpublished observations). These excess chains that remain as free subunits or as partially assembled complexes are largely transported to lysosomes and are rapidly degraded. Subsequent studies on a  $\zeta$ -deficient T cell hybridoma variant, MA5.8, revealed that the  $\zeta$  chain determines the normal post-Golgi fate of the CD3/Ti complex (Sussman et al., 1988a). The  $\zeta$ -minus complexes formed in this cell line are transported normally from the ER to the Golgi. However, the majority of these protein complexes are then transported to lysosomes and degraded while very few complexes reach the cell surface.

Having seen that  $\zeta$ -minus complexes of the TCR surprisingly exhibit normal transport from the ER to the Golgi, but that ER-Golgi transport is highly conformation-dependent in several systems, we wanted to define more precisely the



**Figure 9.** Electron microscopic localization of the CD3-ε chain in 2B4 and 21.2.2 cells. Cells were fixed, permeabilized with saponin, and incubated with 500A2 followed by peroxidase-conjugated, goat anti-hamster IgG. After reaction with diaminobenzidine hydrochloride/ $H_2O_2$ , cells were prepared for electron microscopy using a preembedding immunoperoxidase method as described in Materials and

components of the TCR that are necessary for movement out of the ER. The present studies used the 21.2.2  $\beta$ -deficient T cell hybridoma line which was generated by repetitive subcloning of the 2B4 parent cell line (Sussman et al., 1988b). This cell line expresses no TCR subunits on the cell surface (Fig. 1) despite the fact that the CD3 chains,  $\delta$ ,  $\epsilon$ , and  $\gamma$ , and the  $\zeta$  chain are all synthesized in normal amounts and are partially assembled immediately after synthesis (Fig. 2). The partial complexes that are detectable in 21.2.2 only include  $\delta$ - $\epsilon$ - $\gamma$  while no associated  $\alpha$  or  $\zeta$  chains are observed, presumably because of the lack of  $\beta$ . Direct immunoprecipitation of  $\zeta$  reveals its presence on the surface of 2B4 cells, but not on 21.2.2 cells. We can follow the processing fate of CD3- $\delta$  and - $\gamma$ , however, because they are glycosylated. They are never transported to the Golgi system nor do they accumulate in the *cis*-Golgi stacks as analyzed by digestion of carbohydrate chains with Endo H and Endo D (Figs. 3 and 4, Table I). The Golgi system and the cellular machinery required for intracellular transport of proteins are intact and functional in 21.2.2 cells, however, because we see normal carbohydrate processing of the transferrin receptor in these cells (Fig. 4). Taken together, the data suggest that when the  $\beta$  chain of the TCR is not expressed, the resulting partial complexes of TCR chains fail to move to the Golgi and are retained in a pre-Golgi compartment. We have done some preliminary experiments to show that the well-characterized luminal ER protein, BiP (Haas and Wabl, 1983; Bole et al., 1986; Munro and Pelham, 1986), is not involved in the retention of TCR chains in the ER of 21.2.2 cells (Chen, C., unpublished observations). We were unable to detect an association of BiP with any TCR chains coprecipitated by an anti- $\epsilon$  antibody. In addition, an anti-BiP antibody (kindly provided by Dr. D. Bole) did not coprecipitate any detectable TCR chains.

The fate of the CD3- $\delta$  chain in the pre-Golgi compartment of 21.2.2 cells is strikingly different from that of the other chains we can examine in 21.2.2 (CD3- $\gamma$ , CD3- $\epsilon$ ,  $\zeta$ ). Only the  $\delta$  chain is rapidly degraded in these cells ( $t_{1/2} = 45$ – $60$  min) while the other chains are extremely stable, showing no significant degradation over a 7-h period (Fig. 5). We know that the loss of  $\delta$  is not due to secretion into the medium or to loss of reactivity of the  $\delta$  chain with the anti- $\delta$  antibody. Interestingly, this pre-Golgi degradative system in T cells cannot be inhibited by a variety of lysosomal inhibitors, including  $\text{NH}_4\text{Cl}$ , MME, chloroquine, leupeptin, and monensin (Figs. 6 and 7). The data are analogous to the recent findings of pre-Golgi degradation in stable fibroblast transfectants expressing the TCR- $\alpha$  and/or - $\beta$  chains (Lippincott-Schwartz et al., 1988). We are currently using electron microscopy to attempt to localize the site of pre-Golgi  $\delta$  degradation in 21.2.2 cells more precisely. All of our data suggest that it is occurring either within or closely related to the ER. These data include lack of any Golgi-associated carbohydrate processing of  $\delta$  and the steady-state accumulation of as-

sociated CD3- $\epsilon$  chains in the ER and transitional elements of 21.2.2 cells (Fig. 9). Whether or not  $\epsilon$ ,  $\gamma$ , and  $\zeta$  accumulate in the same compartment as the site of  $\delta$  degradation requires further morphological characterization.

Two possibilities exist for the selective degradation of TCR chains in 21.2.2. First, if the site of degradation is the ER itself, we would expect all newly synthesized proteins to pass through this pre-Golgi proteolytic compartment. Proteins that fail to negotiate ER to Golgi transport would be trapped within this compartment and thereby subjected to degradation. We would then hypothesize that the TCR chains in 21.2.2 are intrinsically differentially sensitive to ER proteases, perhaps due to structural targets for ER degradative enzymes that are present only on  $\delta$ , or to conformational differences among the proteins that allow  $\delta$  to be more accessible to proteolytic cleavage. The latter possibility is more likely in light of the fact that the CD3- $\delta$  and - $\gamma$  chains share sequence similarities at both the DNA and protein levels (Krissansen et al., 1986; Gold et al., 1987). Alternatively, proteins that fail to reach the Golgi and are retained stably in the ER may be sorted from those that fail to reach the Golgi but are rapidly degraded. Such sorting may be to different subregions within the ER or to a distinct, but as yet undefined, degradative organelle. In the case of 21.2.2, only  $\delta$  would undergo selective diversion to such a degradative compartment. For  $\delta$  to be selectively degraded by being sorted to a degradative organelle, a mechanism must exist for dissociating  $\delta$  from the already formed  $\delta$ - $\epsilon$ - $\gamma$  complex. Whether such dissociation occurs and, if so, by what mechanism needs to be addressed. Recent studies in our laboratory demonstrate that free  $\delta$  is much more rapidly degraded than assembled  $\delta$  (Lippincott-Schwartz, J., unpublished observations). We are currently attempting to determine whether any degradation of assembled chains takes place or whether dissociation is a prerequisite for ER degradation. The results of the study on the fate of  $\epsilon$  when coprecipitated with anti- $\delta$  antibodies suggest that  $\delta$  degradation does not occur at the site of synthesis and assembly of receptor chains. However, we cannot rule out the possibility that failure to coprecipitate  $\epsilon$  with anti- $\delta$  antibodies over time is due to the inability of previously synthesized  $\epsilon$ - $\gamma$  complexes to recombine with newly synthesized  $\delta$  chains. Whatever the underlying explanation for the selective degradation of  $\delta$ , these studies demonstrate that individual proteins when "retained" in the ER can be subjected to markedly different fates. This is true even for individual components of an assembled multisubunit complex. This selectivity likely provides the cell with an unexpected site of specific control in the processing and survival of newly synthesized proteins.

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Methods. (A) An overview of the cytoplasm of 2B4 cells shows the distribution of HRP reaction product in ER cisternae (arrows), along the nuclear envelope, in Golgi cisternae and lysosomes, and on the cell surface (arrowheads). (B) An analogous overview of the cytoplasm of 21.2.2 cells shows positive HRP staining in ER cisternae, along the nuclear envelope, and in transitional elements and vesicles. Note the lack of staining in Golgi cisternae, in lysosomes, and along the cell surface. nu, nucleus; er, endoplasmic reticulum; ly, lysosome; te, transitional element; GC, Golgi complex. Bars, 1  $\mu\text{m}$ .

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