

# Pre-Golgi Degradation of Newly Synthesized T-Cell Antigen Receptor Chains: Intrinsic Sensitivity and the Role of Subunit Assembly

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**Abstract.** The T cell antigen receptor (TCR) is a multisubunit complex composed of at least seven transmembrane chains. The predominant species in most T cells has the composition  $\alpha\beta\gamma\delta\epsilon\zeta_2$ . The roles of subunit assembly in transport out of the ER and in the recently described process of pre-Golgi degradation of newly synthesized TCR chains were analyzed in a T-cell line deficient in the synthesis of  $\delta$  chains ( $\Delta 2$ ) and in COS-1 fibroblasts transfected with genes encoding individual TCR chains. Studies with the  $\delta$ -deficient T-cell line showed that, in the absence of  $\delta$ , the other TCR chains were synthesized at normal rates, but, instead of being transported to the cell surface, they were retained in the ER. Analysis of the fate of TCR chains retained in the ER showed that they were degraded at vastly different rates by a nonlysosomal

pathway. Whereas the  $\alpha$  chains were degraded rapidly,  $\gamma$ ,  $\zeta$ , and  $\epsilon$  were relatively long-lived. To analyze whether this selective degradation was because of different intrinsic susceptibility of the individual chains to degradation or to the formation of resistant oligomers, TCR chains were expressed alone or in combinations in COS-1 fibroblasts. These studies showed that (a) individual TCR chains were degraded at different rates when expressed alone in COS-1 cells, and (b) sensitive chains could be stabilized by coexpression with a resistant chain. Taken together, these observations indicate that both intrinsic sensitivity and subunit assembly play a role in determining the rates at which newly synthesized TCR chains are degraded in the ER.

RECENT studies have demonstrated that cells are capable of preventing the expression of abnormal or incompletely assembled membrane multisubunit complexes on their surface. Mutations that alter the conformation of components of homooligomeric complexes frequently result in their failure to be transported to the cell surface (Kreis and Lodish, 1986; Gething et al., 1986; Copeland et al., 1986). In some cases, these changes completely abolish the ability of the proteins to oligomerize. In other cases, the assembly of oligomers is not affected, but the complexes have abnormal conformations. Likewise, the lack of synthesis of individual chains of membrane heterooligomeric complexes also causes intracellular retention of the remaining chains (Weiss and Stobo, 1984; Furley et al., 1986; Rein et al., 1987; Sussman et al., 1988; Chen et al., 1988). Two basic mechanisms underlie the failure to express incomplete complexes on the cell surface: (a) retention of newly synthesized proteins within the ER (reviewed by Rose and Doms, 1988); and (b) selective degradation of abnormal proteins and protein complexes (Minami et al., 1987; Sussman et al., 1988; Lippincott-Schwartz et al., 1988a; Chen et al., 1988). The presence of such mechanisms implies that cells have evolved

the ability to edit the architecture of newly synthesized proteins and protein complexes. This editing prevents mistaken structures from reaching the cell surface or surviving within the cells.

We have been studying the nature of such structural editing mechanisms using the T-cell antigen receptor (TCR)<sup>1</sup> as a model. The TCR is one of the most complex surface receptors described to date. It is composed of at least seven transmembrane chains (Samelson et al., 1985; Oettgen et al., 1986). In most T cells, the TCR comprises two immunoglobulin-like variable chains,  $\alpha$  and  $\beta$ , which are responsible for the ligand recognition function of the receptor, and five invariant chains, CD3- $\gamma$ ,  $\delta$ ,  $\epsilon$ , and the disulfide-linked homodimer  $\zeta_2$ , which probably function in signal transduction (reviewed by Allison and Lanier, 1987; Clevers et al., 1988; Weissman et al., 1988b). In mature T cells, all of these chains are expressed on the cell surface as a complex with a minimum stoichiometry of  $\alpha\beta\gamma\delta\epsilon\zeta_2$ . An additional pro-

1. *Abbreviations used in this paper:* TCR, T-cell antigen receptor; Tf, transferrin; TRAP, T-cell antigen receptor-associated protein.

tein, T-cell receptor associated protein (TRAP) (Bonifacino et al., 1988b) or CD3- $\omega$  (Petty et al., 1987; Alarcon et al., 1988) has been found to interact transiently with the TCR chains during assembly of the complex in the ER.

Early T-cell precursors in the thymus or mutant T cells that fail to synthesize some of the receptor chains lack expression of the remaining chains on the cell surface, even though these chains are synthesized at normal rates (Weiss and Stobo, 1984; Furley et al., 1986; Sussman et al., 1988; Chen et al., 1988). Two mechanisms exist within T cells to prevent transport of incomplete complexes to the cell surface: delivery of newly synthesized TCR chains to lysosomes (Minami et al., 1987; Sussman et al., 1988) and retention in the ER (Lippincott-Schwartz et al., 1988; Chen et al., 1988). The delivery of incomplete complexes to lysosomes was observed in mutant T cells deficient in the synthesis of the  $\zeta$  chains (Sussman et al., 1988) and in T-cell hybridomas in which the  $\zeta$  chains were synthesized in limiting amounts (Minami et al., 1987). In both of these cell lines,  $\alpha\beta\gamma\delta\epsilon$  complexes were assembled in the ER, transported through the entire Golgi system and rapidly degraded by a process sensitive to inhibitors of lysosomal proteolysis. Immunoelectron microscopy studies have directly demonstrated the presence of TCR chains in lysosomes (Chen et al., 1988). Retention of TCR chains in the ER has been observed in a mutant T cell that fails to synthesize any  $\beta$  chains (Chen et al., 1988) and in fibroblasts transfected with genes encoding the  $\alpha$  and  $\beta$  chains (Lippincott-Schwartz et al., 1988). Despite their failure to be transported to the Golgi system, partial complexes were assembled in these cells. Surprisingly, some of the receptor chains retained in the ER were found to be rapidly and efficiently degraded by a nonlysosomal process, suggesting the existence of a new pathway for the degradation of newly synthesized membrane proteins (Lippincott-Schwartz et al., 1988; Chen et al., 1988).

In the present study, we have examined the relationship between the quaternary structure of receptor complexes, retention in the ER and nonlysosomal degradation. To this end, we have characterized the effect of a new deficiency in the structure of the TCR complex, the lack of CD3- $\delta$  chains, on the fate of the individual receptor subunits. In the absence of CD3- $\delta$ , all of the other TCR chains were synthesized and partially assembled in the ER, but failed to be transported into the Golgi system. The TCR chains retained in the ER were degraded at vastly different rates. While the  $\alpha$  chains were rapidly degraded by a nonlysosomal pathway,  $\epsilon$ ,  $\gamma$ , and  $\zeta$  survived for long periods of time. To understand the mechanism(s) underlying this selectivity for degradation, we examined the turnover rates of free and assembled chains in fibroblasts transfected with genes encoding TCR chains. When expressed alone in transfected fibroblasts, TCR chains were also found to be degraded at different rates, indicating that structural information is contained within each chain that determines their sensitivity to degradation. When expressed in combinations, however, certain subunit interactions led to the stabilization of sensitive chains by virtue of their assembly into oligomeric structures. This was strikingly exemplified by the  $\gamma$  chain, which was rapidly degraded when expressed alone but was protected from degradation when assembled with the  $\epsilon$  chain. Two factors, therefore, appear to determine the different fates of individual TCR chains

retained in the ER: (a) intrinsic sensitivity to degradation, and (b) assembly into stable oligomers.

## Materials and Methods

### Specific Reagents

The following antibodies were used for immunoprecipitation and morphological studies: anti- $\delta$ , affinity-purified anti-peptide antiserum No. 1489 raised in a goat immunized with a COOH-terminal peptide of the mouse  $\delta$  chain (Samelson et al., 1986); anti- $\alpha$  (A2B4-2), monoclonal mouse IgG2a that reacts with a clonotypic determinant of the 2B4- $\alpha$  chain (Samelson et al., 1983; Saito et al., 1987); anti- $\epsilon$ , monoclonal hamster antibodies 145-2C11 (Leo et al., 1987) and 500A2 (Havran et al., 1987) and polyclonal antiserum No. 127 raised in a rabbit immunized with purified mouse  $\epsilon$  (Minami et al., 1987); anti- $\gamma\epsilon$ , polyclonal antiserum No. 125 raised against purified mouse  $\gamma$  and  $\epsilon$  (A. M. Weissman, unpublished observations); anti- $\zeta$ , polyclonal antisera No. 124 and No. 387, raised in rabbits immunized with purified mouse  $\zeta$  (Weissman et al., 1986) or a synthetic peptide comprising amino acids 132 to 144 of the murine  $\zeta$  sequence (Weissman et al., 1988a), respectively. For immunoprecipitation studies, anti- $\alpha$ , - $\epsilon$ , - $\gamma\epsilon$ , and - $\zeta$  antibodies were bound to protein A agarose (Bethesda Research Laboratories, Gaithersburg, MD). Affinity-purified anti- $\delta$  antibodies were immobilized by covalent coupling to CNBr-activated Sepharose beads (Pharmacia Fine Chemicals, Uppsala, Sweden). Transferrin receptors were isolated with human transferrin covalently coupled to CNBr-activated Sepharose. A polyclonal rabbit antiserum that reacts with ER resident proteins (Louvard et al., 1982) was kindly provided by Dr. D. Louvard (Pasteur Institute, Paris, France). Fluorescein-conjugated affinity-purified goat anti-hamster IgG was from Kirkegaard & Perry Laboratories, Inc. (Gaithersburg, MD). Rhodamine-conjugated goat anti-mouse IgG or anti-rabbit IgG were from Cappel Laboratories (Malvern, PA). Brefeldin A was a gift of Sandoz Ltd. (Basel, Switzerland).

### Development of a $\delta$ -Deficient Cell Line

T cells deficient in the expression of the CD3- $\delta$  chains were obtained by repetitive subcloning at limiting dilution of the antigen-specific 2B4 T-cell hybridoma line. Screening for low  $\delta$  expressors was performed by immunoblotting with anti- $\delta$  antibodies. After four rounds of single cell cloning at limiting dilution, a cell line was isolated (called 3.12.29.19) that expressed levels of  $\delta$   $\sim$ 50-fold lower than 2B4 cells. This cell line also expressed very low levels of  $\zeta$  chains. The  $\zeta$  deficiency was corrected by transfection of the  $\zeta$  chain cDNA (Weissman et al., 1988a) inserted into the pFNeo expression vector (Saito et al., 1987). Transfection was accomplished by protoplast fusion as described by Oi et al. (1983) and stable transformants were selected with 1 mg/ml G418 (Gibco Laboratories, Grand Island, NY). Five clones were obtained, four of which expressed the  $\zeta$  chains. One of them, named  $\Delta 2$ , was selected for further analysis. 2B4 and  $\Delta 2$  cells were grown in T-cell medium, as previously described (Samelson and Schwartz, 1983).  $\Delta 2$  cells were treated with 1–2 mM sodium butyrate 16 h before each experiment to enhance the expression of the transfected  $\zeta$  gene as described by Gorman et al. (1983). The addition of sodium butyrate did not cause any effect on the processes analyzed in this study, as previously reported (Lippincott-Schwartz et al., 1988). Under these conditions,  $\Delta 2$  cells displayed  $\sim$ 5% the number of surface receptors found on the parental line 2B4, as determined by flow cytometry using an anti- $\alpha$  antibody (A2B4-2).

### Expression of TCR Chains in COS-1 Cells

For transient expression in COS-1 fibroblasts, cDNAs encoding the 2B4- $\alpha$  and the murine  $\gamma$ ,  $\epsilon$ , and  $\zeta$  chains were cloned into the expression vector pSVL (Pharmacia Fine Chemicals). A PBS plasmid (Stratagene Cloning Systems, La Jolla, CA) containing a 1.6-kb insert encoding the 2B4- $\alpha$  cDNA (provided by Dr. S. M. Hedrick, University of California, San Diego) was cut with Xba I to release a 1.3-kb fragment comprising all of the 2B4- $\alpha$  coding sequences (Chien et al., 1984; Saito et al., 1984; Becker et al., 1985). This fragment was cloned into the Xba I site of pSVL. The orientation of the insert was determined by digestion with Pvu II. A 0.7-kb fragment encoding the mouse CD3- $\gamma$  chain (Krissansen et al., 1987) was cut with Eco RI from a pUC 9 vector (pBI0.AT3 $\gamma$ -1, provided by Dr. M. Crumpton, Imperial Cancer Research Fund, London, England). This fragment was cloned into

the Eco RI site of pGEM-7Zf(+) (Promega Biotec, Madison, WI) to create convenient flanking restriction sites. The  $\gamma$  cDNA was then released with Xba I and Bam HI and ligated to an Xba I–Bam HI digest of pSVL. The mouse CD3- $\epsilon$  cDNA (Gold et al., 1987) was excised as a 1.5-kb fragment from a pGEM-1 vector (pDL1, provided by Dr. D. P. Gold, Medical Biology Institute, La Jolla, California) using Eco RI. The recessed ends were filled in using Klenow fragment and the blunt-ended cDNA was cloned into the Sma I site of pSVL. The orientation was tested with Xho I. A Kpn I to Eco RI fragment of the murine zeta cDNA (Weissman et al., 1988a) was ligated into pGEM-4Z. After cloning this fragment, the vector was cut with Eco RI, the recessed end filled as above and then cut with Xba I (contained within the polylinker). This fragment was isolated and ligated into pSVL, which had been cut with Xba I and Sma I.

Calcium phosphate precipitates containing 20–30  $\mu$ g plasmid DNA/ml-liliter were prepared essentially as described by Gorman et al., 1982. COS-1 cells grown to 80–90% confluency in 150-mm-diam tissue culture dishes were incubated for 16 h at 37°C with 1 ml of calcium phosphate-precipitated DNA and 10 ml DME containing 10% FBS and 0.15 mg/ml gentamicin (COS cell medium). The medium was then replaced by fresh COS cell medium and the cells were grown for an additional 48 h. Sodium butyrate (2 mM) was added to the culture medium 16 h before the experiments, to enhance the expression of the transfected genes (Gorman et al., 1983).

### Metabolic Labeling

T cells or transfected COS-1 cells were preincubated for 15 min at 37°C with methionine- or cysteine-free medium (methionine- or cysteine-free RPMI 1640, Biofluids, Inc., Rockville, MD) containing 5% FBS and 0.15 mg/ml gentamicin. Cells were then labeled for 30 min at 37°C with 0.2–0.5 mCi/ml [<sup>35</sup>S]methionine (Trans-<sup>35</sup>S-label, ICN Radiochemicals, Irvine, CA) in methionine-free medium or 1 mCi/ml [<sup>35</sup>S]cysteine (ICN Radiochemicals) in cysteine-free medium and, when indicated, chased for various periods of time in complete T-cell medium. Labeled proteins were extracted by solubilization in ice-cold 0.5% (wt/vol) Triton X-100, 0.3 M NaCl, 50 mM Tris/HCl, pH 7.4 for 15 min. Insoluble material was removed by centrifugation for 15 min at maximum speed in a microfuge (Beckman Instruments, Inc., Palo Alto, CA) at 4°C. Isolation of labeled TCR chains with anti-TCR antibodies and electrophoretic techniques were as previously described (Samelson et al., 1985). Treatments with endo H (Genzyme, Boston, MA) and endo D (Calbiochem-Behring Corp., San Diego, CA) have also been described before (Chen et al., 1988). Fluorograms were scanned using a densitometer (ultrascan XL; LKB Instruments Inc., Bromma, Sweden).

### Immunofluorescence Microscopy

Surface TCR chains were stained by incubating intact 2B4 or  $\Delta$ 2 cells with monoclonal anti- $\alpha$  (A2B4-2) or anti- $\epsilon$  (500A2) antibodies for 1 h at room temperature followed by rhodamine-conjugated goat anti-mouse IgG (1:100 dilution) or fluorescein-conjugated goat anti-hamster IgG (1:100 dilution), respectively. Excess antibodies were removed by centrifugation in ice-cold PBS. For staining of intracellular TCR chains, 2B4 and  $\Delta$ 2 cells were attached to concanavalin A-coated cover glasses as previously described (Lippincott-Schwartz et al., 1989), fixed with 2% formaldehyde in PBS and permeabilized with 0.1% saponin in PBS. Cells were then incubated with either anti- $\alpha$  (A2B4-2) or anti- $\epsilon$  (500A2) in combination with a polyclonal anti-ER antiserum for 1 h at room temperature. After washing off the unbound antibodies with PBS for 15 min, cells were incubated for 30 min at room temperature with either fluorescein-conjugated goat anti-mouse IgG or fluorescein-conjugated goat anti-hamster IgG together with rhodamine-conjugated goat anti-rabbit IgG at 1:100 dilutions. After an additional 15 min wash in PBS, coverslips were mounted on glass slides using fluoromount G (Southern Biotechnology Associates, Birmingham, AL) and examined with an inverted microscope (ICM405; Carl Zeiss, Inc., Thornwood, NY).

## Results

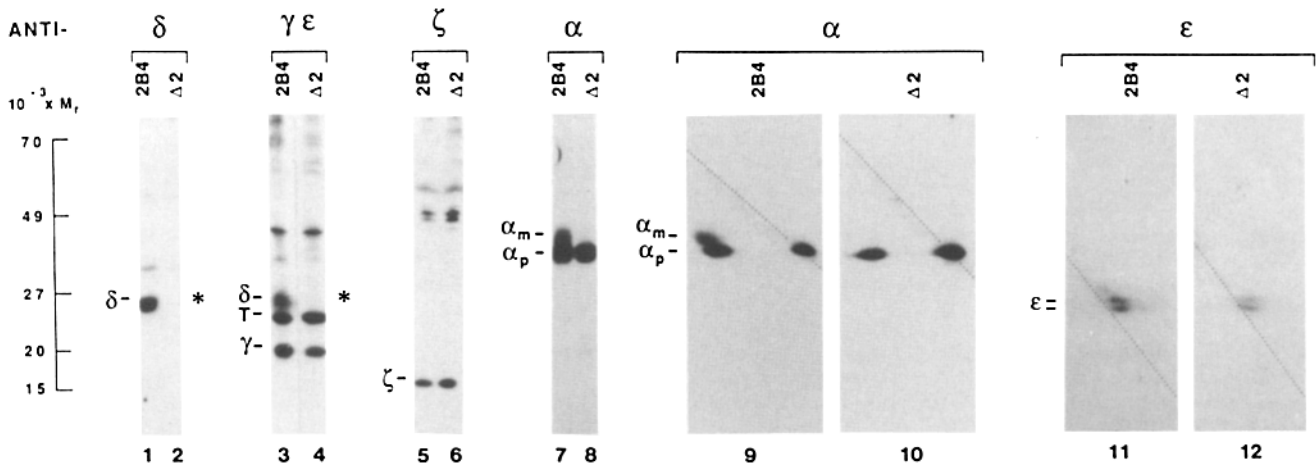
### TCR Complexes Lacking the $\delta$ Chain Are Retained in the ER

Previous studies have shown that incompletely assembled TCR complexes fail to be transported to the cell surface. In-

terestingly, different structural defects result in different intracellular fates for the incomplete receptors. When cells fail to synthesize one of the components of the  $\alpha\beta$  clonotypic heterodimer, the other chains are retained in the ER (Chen et al., 1988; Hannum et al., 1987; Furley et al., 1987). Lack of synthesis of the  $\zeta$  homodimer, on the other hand, results in normal transport of the incomplete complexes from the ER into the Golgi system, but these complexes are eventually delivered to lysosomes for degradation (Minami et al., 1987; Sussman et al., 1988). The role of the other components of the TCR complex (i.e., the invariant  $\gamma$ ,  $\delta$ , and  $\epsilon$  chains) in intracellular transport has yet to be addressed. These proteins have been shown to form  $\gamma\delta\epsilon$  subcomplexes (referred to as “CD3”) regardless of the presence or absence of the other receptor chains (Berkhout et al., 1988; Bonifacino et al., 1988a). In addition, several lines of evidence indicate that these proteins are closely related to each other. Genes encoding these receptor chains have been localized to the same chromosomal region and sequence analysis has revealed a considerable degree of homology, suggesting that they may have arisen from a common ancestral gene (Clevers et al., 1988).

To assess the importance of the components of the CD3 complex in the intracellular transport and eventual fate of TCR chains, we sought to derive a T-cell line deficient in the synthesis of a CD3 chain. A murine T-cell hybridoma line named  $\Delta$ 2, that had steady state levels of  $\delta$  chains  $\sim$ 50-fold lower than the parental T-cell line 2B4, was identified and isolated as described in Materials and Methods. To analyze whether the low content of  $\delta$  chains was because of decreased biosynthesis, 2B4 and  $\Delta$ 2 cells were metabolically labeled with [<sup>35</sup>S]methionine or [<sup>35</sup>S]cysteine, and the TCR chains were isolated by immunoprecipitation with specific antibodies (Fig. 1). Analysis of anti- $\delta$  immunoprecipitates by one-dimensional SDS-PAGE revealed that  $\Delta$ 2 cells synthesized very low levels of CD3- $\delta$  chains as compared to the parental T-cell line 2B4 (Fig. 1, lanes 1 and 2). Analysis of the same samples by two-dimensional nonreducing/reducing SDS-PAGE showed that  $\Delta$ 2 cells synthesized  $\sim$ 3% the amount of  $\delta$  chains made by 2B4 cells (data not shown). In contrast to  $\delta$ , synthesis of the  $\gamma$ ,  $\zeta$ ,  $\alpha$ , and  $\epsilon$  chains in  $\Delta$ 2 cells was comparable to 2B4 cells (Fig. 1). Synthesis of  $\beta$  chains could not be directly assessed for lack of anti- $\beta$  antibodies. However, the  $\alpha$  chains were found to form a disulfide-linked dimer both in 2B4 and  $\Delta$ 2 cells (Fig. 1, lanes 9 and 10). Since the 2B4- $\alpha$  chains do not appear to form homodimers (Bonifacino et al., 1988a), this observation indirectly demonstrated the synthesis of  $\beta$  chains in  $\Delta$ 2 cells. Immunoprecipitation with anti- $\gamma\epsilon$  antibodies also revealed similar amounts of the transiently associated protein TRAP in 2B4 and  $\Delta$ 2 cells (Fig. 1, lanes 3 and 4). Consistent with the low level of  $\delta$  biosynthesis in  $\Delta$ 2 cells (Fig. 1, lanes 1 and 2), no  $\delta$  chains were detected in association with  $\gamma$  and  $\epsilon$  (Fig. 1, lanes 3 and 4).

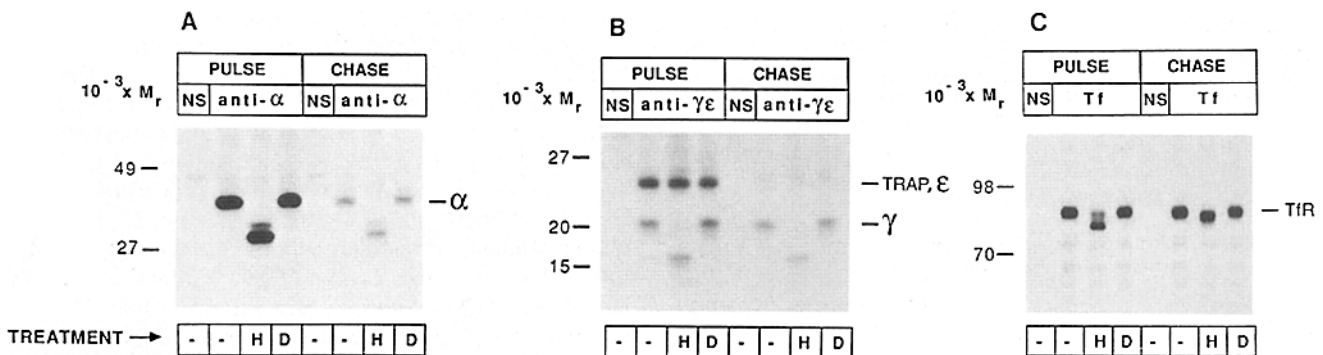
The appearance of the mature  $\alpha$  chains in 2B4 but not in  $\Delta$ 2 cells after a 30-min pulse with [<sup>35</sup>S]methionine (Fig. 1, lanes 7–10), suggested abnormal processing of their carbohydrate chains in the  $\delta$ -deficient T cells. In 2B4 cells, the  $\alpha$  chains are synthesized as an  $M_r$  38,000 precursor that is rapidly processed into a  $M_r$  42,000–44,000 form in the Golgi system (Bonifacino et al., 1988b; Lippincott-Schwartz et al., 1988). Likewise, the single N-linked oligosaccharide



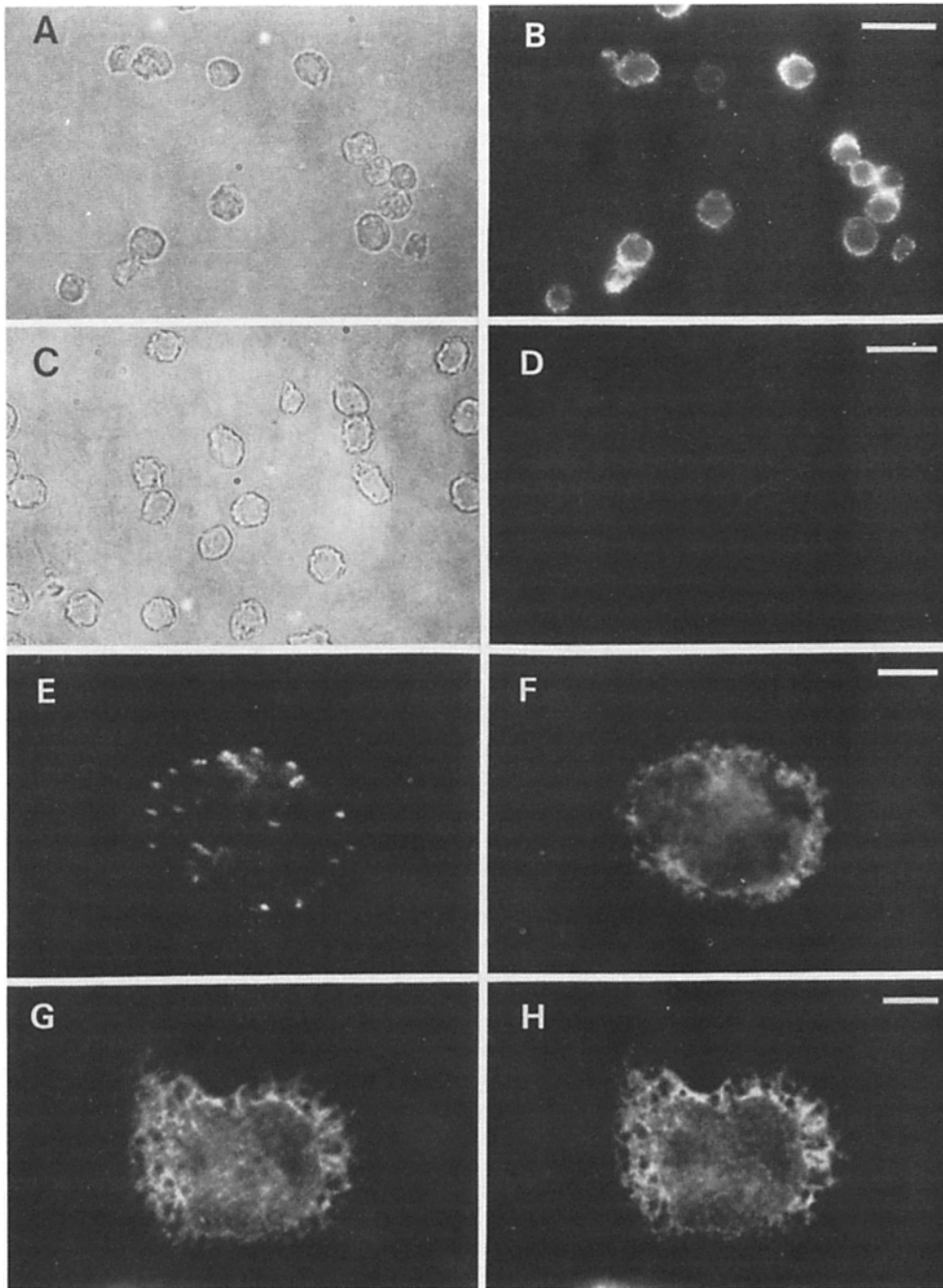
**Figure 1.** Biosynthesis of TCR chains in 2B4 and  $\Delta 2$  cells. 2B4 and  $\Delta 2$  cells were pulse labeled for 30 min at 37°C with [<sup>35</sup>S]methionine (lanes 1–10), or [<sup>35</sup>S]cysteine (lanes 11 and 12), extracted with 0.5% Triton X-100, and TCR chains were isolated with anti- $\delta$  (anti-serum No. 1489; lanes 1 and 2), anti- $\gamma\epsilon$  (anti-serum No. 125, lanes 3 and 4), anti- $\zeta$  (anti-serum No. 387; lanes 5 and 6), anti- $\alpha$  (A2B4-2, lanes 7–10), and anti- $\epsilon$  (anti-serum No. 127; lanes 11 and 12). Immunoprecipitates were analyzed by either one-dimensional SDS-PAGE under reducing conditions (lanes 1–8) or two-dimensional nonreducing/reducing SDS-PAGE (lanes 9–12; nonreducing, left to right; reducing, top to bottom). The positions of molecular weight markers (expressed as  $10^{-3} \times M_r$ ) appear to the left of the figure. The positions of TCR chains and associated proteins are indicated. Lanes 1 and 2, notice the absence of the  $\delta$  chain in  $\Delta 2$  cells (\*). Lanes 3 and 4, the anti- $\gamma\epsilon$  antibody immunoprecipitates  $\gamma$ , TRAP, and  $\delta$  from 2B4 cells. The  $\epsilon$  chain in T cells is poorly labeled with [<sup>35</sup>S]methionine. Notice the absence of co-precipitated  $\delta$  in  $\Delta 2$  cells (\*). Lanes 5 and 6. The anti- $\zeta$  antibody immunoprecipitates comparable amounts of  $\zeta$  from both cell lines. Lanes 7 and 8, notice the appearance of the mature  $\alpha$  chain ( $\alpha_m$ ) in 2B4 but not in  $\Delta 2$  cells. Lanes 9 and 10, the dotted line marks the position of the diagonal. Nondisulfide linked  $\alpha$  appears on the diagonal. Disulfide-linked  $\alpha\beta$  dimers appear below the diagonal. Lanes 11 and 12. The dotted line marks the position of the diagonal. The  $\epsilon$  chain, labeled with [<sup>35</sup>S]cysteine, appears as a doublet above the diagonal. T, TRAP;  $\alpha_p$ ,  $\alpha$  chain precursor;  $\alpha_m$ , mature  $\alpha$  chain.

chain of the  $\gamma$  subunit is terminally processed upon transfer into the Golgi system, even though this maturation does not result in a change in its electrophoretic mobility (Chen et al., 1988). To analyze whether the  $\alpha$  and  $\gamma$  chains in  $\Delta 2$  cells were transported into the Golgi system, cells were pulse-labeled for 30 min with [<sup>35</sup>S]methionine or pulsed for 30 min and chased for 90 min. The  $\alpha$  and  $\gamma$  chains were then isolated with specific antibodies, and the structure of N-linked oligosaccharide chains was examined by digestion with endoglycosaminidases H and D (endo H and endo D). Endo H

cleaves “high mannose” chains and has been used to monitor transport into the medial Golgi cisternae (Tarentino and Maley, 1974; Kornfeld and Kornfeld, 1985). Endo D cleaves  $\text{Man}_5\text{GlcNAc}_2$  intermediates generated during processing of high mannose chains in *cis* Golgi cisternae (Koide and Muramatsu, 1974). As expected from their location in the ER early after synthesis,  $\alpha$  and  $\gamma$  chains in  $\Delta 2$  cells were sensitive to endo H and resistant to endo D after a 30 min pulse (Fig. 2, A and B, PULSE). In contrast to the processing of  $\alpha$  and  $\gamma$  carbohydrates observed in 2B4 cells (Bonifacino et



**Figure 2.** Lack of Golgi processing of TCR chains in  $\Delta 2$  cells.  $\Delta 2$  cells were pulse labeled for 30 min at 37°C with [<sup>35</sup>S]methionine and then, they were either placed on ice (PULSE) or chased for 90 min at 37°C in complete T-cell medium (CHASE). TCR- $\alpha$  (A) and - $\gamma$  chains (B) and transferrin receptors (C) were isolated from detergent-solubilized cells using anti- $\alpha$  (A2B4-2) and anti- $\gamma\epsilon$  (No. 125) antibodies or transferrin (Tf)-Sepharose, respectively, as indicated in the figure. Nonspecific precipitation controls (NS) were performed using the monoclonal antibody F23 (Staerz et al., 1985) (for  $\alpha$ ); nonimmune serum (for  $\gamma$ ) or protein A-Sepharose (for transferrin receptors). Precipitates were then either not treated (–) or treated with endo H (H) or endo D (D) before analysis by one-dimensional SDS-PAGE under reducing conditions. The positions of molecular weight markers (in  $10^{-3} \times M_r$ ) appear to the left. The positions of TCR chains and the Tf receptor are indicated on the right of the respective panels.



**Figure 3.** Localization of TCR chains in 2B4 and  $\Delta 2$  cells by immunofluorescence microscopy. *A-D*, intact 2B4 (*A-B*) and  $\Delta 2$  cells (*C-D*) were surface-stained with the anti- $\epsilon$  antibody 500A2 and fluorescein-conjugated goat anti-hamster IgG. Cells were examined by phase-contrast (*A* and *C*) and fluorescence microscopy (*B* and *D*). Notice the absence of staining on the surface of  $\Delta 2$  cells (*D*). Bars, 20  $\mu\text{m}$ . *E-H*, 2B4 (*E-F*) and  $\Delta 2$  cells (*G-H*) were fixed with formaldehyde, permeabilized with saponin, and incubated with a combination of the hamster anti- $\epsilon$  antibody 500A2 and a rabbit anti-ER antibody, followed by fluorescein-conjugated goat anti-hamster IgG and rhodamine-conjugated goat anti-rabbit IgG. *E* and *G*, fluorescein channel (anti- $\epsilon$ ). *F* and *H*, rhodamine channel (anti-ER). Notice the localization of  $\epsilon$  in the ER of  $\Delta 2$  (*G* and *H*) but not 2B4 cells (*E* and *F*). Bars, 2.5  $\mu\text{m}$ .

al., 1988b; Chen et al., 1988),  $\alpha$  and  $\gamma$  chains in  $\Delta 2$  cells remained sensitive to endo H after a 90 min chase (Fig. 2, *A* and *B*, CHASE), indicating that they failed to be transported into the medial Golgi system. In addition,  $\alpha$  and  $\gamma$  remained resistant to endo D (Fig. 2, *A* and *B*); therefore, we concluded that these chains did not accumulate in *cis* Golgi cisternae. Normal processing of the transferrin receptor in  $\Delta 2$  cells indicated that the lack of processing of TCR chains was not the result of a general defect in carbohydrate processing or intracellular traffic (Fig. 2 *C*).

In agreement with these biochemical data suggesting lack of transport of TCR chains into the Golgi system of  $\Delta 2$  cells,

staining of intact 2B4 and  $\Delta 2$  cells with anti- $\epsilon$  (Fig. 3, *A-D*) or anti- $\alpha$  antibodies (not shown) followed by fluorescently labeled second antibodies revealed the presence of surface receptors in 2B4 but not in  $\Delta 2$  cells. When 2B4 cells were permeabilized before immunofluorescent staining, intracellular TCR chains were found in cytoplasmic vesicles (Fig. 3 *E*), consistent with their localization in the Golgi system, lysosomes and endosomes, as previously shown (Chen et al., 1988). By contrast, in  $\Delta 2$  cells both  $\epsilon$  and  $\alpha$  chains were localized to a reticular network identified as the ER by colocalization with an ER marker (Fig. 3, *G* and *H*). Taken together, these results indicate that, in the absence of the

CD3- $\delta$  chains, other TCR components are synthesized at normal rates but instead of being transported to the cell surface; however, they are retained in the ER.

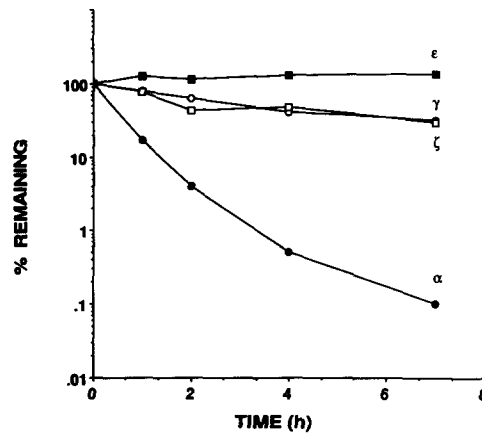
### TCR Chains in $\Delta 2$ Cells Are Degraded at Different Rates by a Nonlysosomal Pathway

The fate of the TCR chains retained in the ER of  $\Delta 2$  cells was analyzed by pulse-chase labeling and direct immunoprecipitation with specific anti-TCR antibodies, as shown in Fig. 4. These experiments showed that whereas the  $\alpha$  chains were rapidly degraded ( $t_{1/2} \sim 30$  min),  $\gamma$  and  $\zeta$  were more stable ( $t_{1/2}$  4–8 h). The  $\epsilon$  chains were the most stable of the TCR chains ( $t_{1/2} > 8$  h).

We next examined the characteristics of the rapid degradation of  $\alpha$  chains in  $\Delta 2$  cells. Our previous studies have shown that newly synthesized TCR chains can be degraded by two different pathways: lysosomal (Minami et al., 1988; Sussman et al., 1988; Lippincott-Schwartz et al., 1988) and nonlysosomal (Lippincott-Schwartz et al., 1988; Chen et al., 1988). Several characteristics of the degradation of  $\alpha$  chains in  $\Delta 2$  cells suggested that it occurred by a nonlysosomal pathway. First, as shown above, degradation of the  $\alpha$  chains occurred without processing of oligosaccharide chains in the Golgi system (Fig. 2). This would suggest that the TCR chains are not transported to lysosomes, since the only well established route of transit of newly synthesized membrane proteins to lysosomes passes through the entire Golgi system (Barriocanal et al., 1986; Green et al., 1987; Sussman et al., 1988). Second, degradation was selective for particular TCR chains (Fig. 4). This selectivity is a hallmark of pre-Golgi, nonlysosomal degradation (Chen et al., 1988) and is in contrast to the rather indiscriminate destruction of TCR chains in lysosomes (Minami et al., 1988). Finally, inhibitors of lysosomal proteolysis, including  $\text{NH}_4\text{Cl}$  and leupeptin, and the metabolic poisons  $\text{NaN}_3$  and  $\text{NaF}$ , failed to block degradation of  $\alpha$  in these cells (Table I), even though they inhibit degradation of TCR chains in T-cell hybridomas or  $\zeta$ -deficient T cells (Lippincott-Schwartz et al., 1988; Chen et al., 1988; Sussman et al., 1988). In addition, degradation of  $\alpha$  chains in  $\Delta 2$  cells displayed the characteristic sensitivity to low temperatures previously reported for the pre-Golgi, nonlysosomal pathway (Lippincott-Schwartz et al., 1988).

### Assembled and Unassembled $\gamma$ Chains Are Degraded at Different Rates in $\Delta 2$ Cells

The strikingly different rates at which TCR chains are degraded in  $\Delta 2$  cells could be due to one or both of two possible causes. Each TCR chain could have a different intrinsic susceptibility to pre-Golgi degradation. Alternatively, the formation of particular oligomeric structures could protect otherwise sensitive TCR chains from degradation. As a first step to distinguish these possibilities, we compared the rates of disappearance of assembled and unassembled  $\gamma$  chains in  $\Delta 2$  cells. In these experiments, the amount of assembled  $\gamma$  chains was quantitated using the monoclonal anti- $\epsilon$  antibody 2C11, which causes minimal disruption of the CD3 complex (Bonifacino et al., 1988a). Unassembled  $\gamma$  chains were isolated with a polyclonal anti- $\gamma\epsilon$  antibody after removal of the assembled  $\gamma$  with anti- $\epsilon$ . After a 30-min labeling period, the majority (87%) of  $\gamma$  chains were assembled into  $\epsilon$ -containing



**Figure 4.** Fates of TCR chains in  $\Delta 2$  cells.  $\Delta 2$  cells were pulse labeled for 30 min at 37°C with [ $^{35}\text{S}$ ]methionine as indicated in the Materials and Methods section. Cells were then chased for different periods of time at 37°C with complete T-cell medium. At the times indicated, cells were collected by centrifugation and frozen at  $-70^\circ\text{C}$ . The cell pellets were then thawed at 4°C and extracted with lysis buffer. The TCR chains were isolated with anti- $\epsilon$  (2C11), anti- $\zeta$  (No. 124), anti- $\gamma\epsilon$  (No. 125) and anti- $\alpha$  (A2B4-2) antibodies. Immunoprecipitates were analyzed by one-dimensional SDS-PAGE under reducing conditions (for  $\gamma$  and  $\alpha$ ) or two-dimensional non-reducing-reducing SDS-PAGE (for  $\epsilon$  and  $\zeta$ ). The relative amount of each TCR chain (with respect to time 0) was determined by densitometric scanning of autoradiograms. The results shown are representative of at least two independent determinations for each receptor chain. ■  $\epsilon$ ; □  $\zeta$ ; ○  $\gamma$ ; ●  $\alpha$ .

complexes. Examination of the fate of assembled and unassembled  $\gamma$  chains revealed a difference in their initial rates of disappearance. Whereas assembled  $\gamma$  was relatively stable ( $t_{1/2}$  7 h), unassembled  $\gamma$  disappeared with a  $t_{1/2}$  of less than 2 h (data not shown). The loss of unassembled  $\gamma$  chains could result from either an increased sensitivity to pre-Golgi degradation of free with respect to assembled  $\gamma$  or to associa-

**Table I.** Characteristics of  $\alpha$  and  $\gamma$  Degradation in  $\Delta 2$  and COS-1 Cells

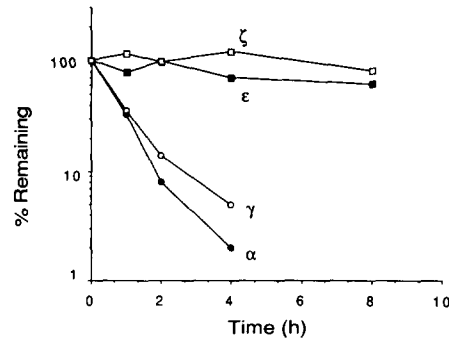
Treatment	% Inhibition		
	$\alpha \Delta 2$	$\alpha$ COS-1	$\gamma$ COS-1
(a) None (control)	0	0	0
(b) $\text{NH}_4\text{Cl}$ , 50 mM	0	6	9
(c) Leupeptin, 0.1 mg/ml	12	0	0
(d) $\text{NaN}_3$ , 0.1%/NaF, 20 mM	15	18	0
(e) 16°C	89	85	95

$\Delta 2$  or COS-1 cells transfected with the 2B4  $\alpha$  or  $\gamma$  cDNAs were either not treated (a, d, and e) or incubated for 1 h at 37°C with 50 mM  $\text{NH}_4\text{Cl}$  (b) or 0.1 mg/ml leupeptin (c) before labeling for 20 min at 37°C with [ $^{35}\text{S}$ ]methionine. Labeled cells were chased for 0, 2, and 5 h at 37°C (a–d) or 16°C (e) in complete T-cell medium.  $\text{NH}_4\text{Cl}$  or leupeptin at the concentrations mentioned above (b and c) or a mixture of 0.1%  $\text{NaN}_3$  and 20 mM NaF (d) were added to the chase medium, as indicated in the table. The  $\alpha$  and  $\gamma$  chains were isolated by immunoprecipitation with the monoclonal anti- $\alpha$  antibody A2B4 or the polyclonal anti- $\gamma\epsilon$  antiserum No. 125, respectively. After separation by reducing SDS-PAGE, the intensity of protein bands was quantitated by densitometric scanning of autoradiograms. The rates of degradation were calculated assuming first order kinetics and the percent inhibition by each treatment was determined by comparison to the rate of degradation in the control cells (a).

tion of unassembled  $\gamma$  with  $\epsilon$ . Because of the uncertainties inherent to this type of experiment, we decided to examine the fates of TCR chains expressed alone or in combinations in transfected fibroblasts, to more precisely define the role of subunit assembly in pre-Golgi degradation.

### Selective Degradation of TCR Chains Expressed in Fibroblasts

To assess whether individual receptor chains have structural information that determines retention in the ER and/or sensitivity to nonlysosomal degradation, we analyzed the fate of the  $\alpha$ ,  $\gamma$ ,  $\epsilon$ , and  $\zeta$  chains expressed individually in fibroblasts. To this end, cDNAs encoding TCR chains were inserted into pSVL, an expression vector containing the SV40 late promoter, origin of replication and polyadenylation signal, and these plasmids were used to transfect COS-1 cells. When expressed in COS-1 cells, newly synthesized  $\alpha$  and  $\gamma$  chains failed to acquire Endo H resistance and Endo D sensitivity, consistent with their being retained in the ER (Fig. 5). The intracellular localization of two other TCR chains,  $\epsilon$  and  $\zeta$ , in COS-1 cells, however, was more difficult to determine since these chains do not have N-linked oligosaccharides and could not be localized morphologically with the reagents available. Since we were interested in studying the fate of each of the TCR chains when retained in the ER, we needed assurance that  $\epsilon$  and  $\zeta$  were not transported out of the ER to the Golgi system. For this purpose, we used the antibiotic brefeldin A, which has been shown to block the transport of newly synthesized proteins out of the ER (Takatsuki and Tamura, 1985; Misumi et al., 1986; Lippincott-Schwartz et al., 1989) without affecting the efficiency or selectivity of pre-Golgi system degradation (Lippincott-Schwartz et al., 1989). The transfected cells were pulse-labeled for 30 min with [ $^{35}$ S]methionine and chased for different periods of time at 37°C in the presence of brefeldin A. As shown in Fig. 6,  $\alpha$  chains were degraded, while  $\epsilon$  and  $\zeta$  survived long term. In contrast to what was observed in  $\Delta 2$  cells (Fig. 4),  $\gamma$  chains were almost as rapidly degraded as  $\alpha$  chains (Fig. 6). Similar results to these were obtained in the absence of brefeldin A, confirming that this drug had no effect on degradation (data not shown). The experiment shown in Fig. 7 demonstrated that the loss of  $\alpha$  and  $\gamma$  chains observed after a long chase was probably not due to the formation of aggregates that were insoluble in Triton X-100, since no additional chains could be extracted with SDS. In addition, TCR

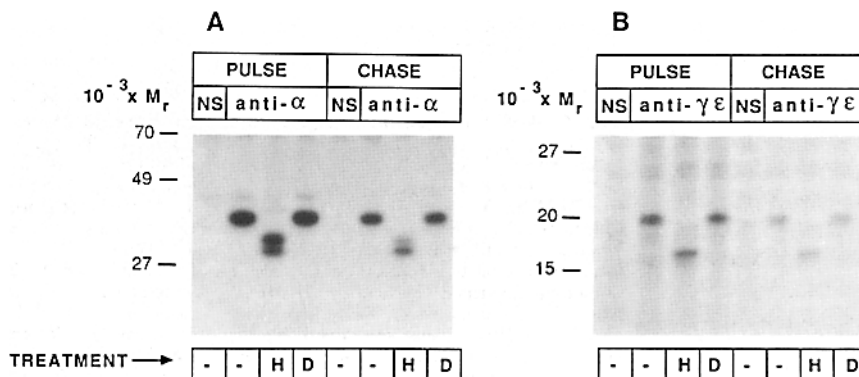


**Figure 6.** Different fates of TCR chains in transfected COS-1 cells. COS-1 cells, expressing the mouse  $\epsilon$ ,  $\zeta$ ,  $\alpha$ , or  $\gamma$  chains, were preincubated for 30 min at 37°C with [ $^{35}$ S]methionine and then incubated for different periods of time in complete T-cell medium, all in the continuous presence of 5  $\mu$ g/ml brefeldin A. TCR chains were isolated by immunoprecipitation of detergent-solubilized cells with anti- $\epsilon$  (No. 127), anti- $\zeta$  (No. 124), anti- $\alpha$  (A2B4-2), or anti- $\gamma$  (No. 125). Samples were analyzed by one-dimensional SDS-PAGE under reducing conditions. The amount of each chain remaining at every time point was determined by densitometric scanning of autoradiograms. The experiment was repeated two times in the absence of brefeldin A, with similar results. ■,  $\epsilon$ ; □,  $\zeta$ ; ○,  $\gamma$ ; ●,  $\alpha$ .

chains were not shed into the extracellular medium, as demonstrated by the failure to isolate any chains from the culture supernatant (Fig. 7). The loss of  $\alpha$  and  $\gamma$  in COS-1 cells had all the characteristics of the pre-Golgi nonlysosomal degradation described earlier (Lippincott-Schwartz et al., 1988; Chen et al., 1988), including the lack of inhibition by  $\text{NH}_4\text{Cl}$ , leupeptin,  $\text{NaN}_3/\text{NaF}$  and the inhibition at 16°C (Table I). These observations indicated that each TCR chain has an intrinsic sensitivity to degradation in the ER that is manifested when each chain is expressed alone. However, the different fates of  $\gamma$  when assembled into oligomers, as in  $\Delta 2$  cells (Fig. 4), and when expressed alone, as in COS-1 cells (Fig. 6), suggested that oligomerization may modify the rate of pre-Golgi degradation.

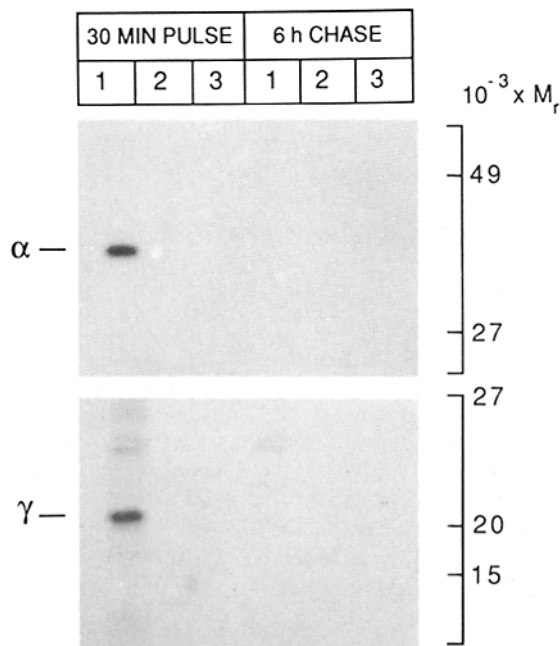
### Stabilization of the $\gamma$ Chains by Assembly with $\epsilon$

To test directly whether assembly of TCR chains can affect their susceptibility to pre-Golgi system degradation, the fates of  $\gamma$  and  $\epsilon$  chains expressed either individually or together in COS-1 cells were examined. As shown in Fig. 8 A, the



**Figure 5.** Lack of Golgi processing of TCR chains in transfected COS-1 cells. The processing of N-linked oligosaccharide chains in  $\alpha$  (A) and  $\gamma$  chains (B) expressed in COS-1 cells was analyzed as described in legend to Fig. 2 for  $\Delta 2$  cells.





**Figure 7.**  $\alpha$  and  $\gamma$  chains in transfected COS cells do not become insoluble in Triton X-100 and are not shed into the culture medium. COS cells expressing  $\alpha$  (top) or  $\gamma$  (bottom) were metabolically labeled with [ $^{35}$ S]methionine for 30 min at 37°C. After removing the labeling medium and adding complete T-cell medium, cells were either placed on ice (PULSE) or incubated for 6 h at 37°C (CHASE). Cells and supernatants were collected separately. Cell pellets were first extracted for 15 min at 4°C with regular lysis buffer (0.5% Triton X-100, 0.3 M NaCl, 50 mM Tris/HCl, pH 7.4). The insoluble material was reextracted for an additional 15 min at 4°C with lysis buffer containing 0.2% SDS. The Triton X-100 extracts (lanes 1), SDS extracts (lanes 2) and supernatants (lanes 3) were subjected to immunoprecipitation with anti- $\alpha$  (top) or anti- $\gamma$  antibodies (bottom). The positions of molecular weight markers (in  $10^{-3} \times M_r$ ) appear to the right.

polyclonal anti- $\gamma$  antisera No. 125 could be used to immunoprecipitate the  $\gamma$  and  $\epsilon$  chains regardless of the state of association. Immunoprecipitation analysis of doubly transfected cells with this antibody revealed similar levels of expression of  $\gamma$  and  $\epsilon$  (Fig. 8 A, lane 8). These chains were efficiently assembled in COS-1 cells, as demonstrated by their co-precipitation using the monoclonal anti- $\epsilon$  antibodies 2C11 (Fig. 8 A, lane 9) or 500A2 (not shown). Interestingly, in the absence of the  $\gamma$  chains, the  $\epsilon$  chains were very poorly immunoprecipitated by both monoclonal anti- $\epsilon$  antibodies 2C11 (Fig. 8 A, lane 6) and 500A2 (not shown). This observation suggested that these antibodies recognize a conformational state of the  $\epsilon$  molecule that is favored by the assembly of a  $\gamma$   $\epsilon$  complex.

Analysis of the time course of degradation revealed a dramatic stabilization of the  $\gamma$  chains by the simultaneous expression of  $\epsilon$  (Fig. 8, B and C). This observation demonstrated that chains that are sensitive to pre-Golgi, nonlysosomal degradation can be rendered resistant by assembly with intrinsically stable chains. In contrast to these results, when  $\gamma$  and 2B4- $\alpha$  chains were co-expressed in COS-1 cells, they did not form stable complexes and their rates of degradation were the same as when they were expressed alone

(data not shown). Taken together, these studies suggest that intrinsic sensitivity of individual TCR chains to pre-Golgi degradation and the ability of these chains to oligomerize both play important roles in determining the rates at which these chains are degraded while retained in the ER.

## Discussion

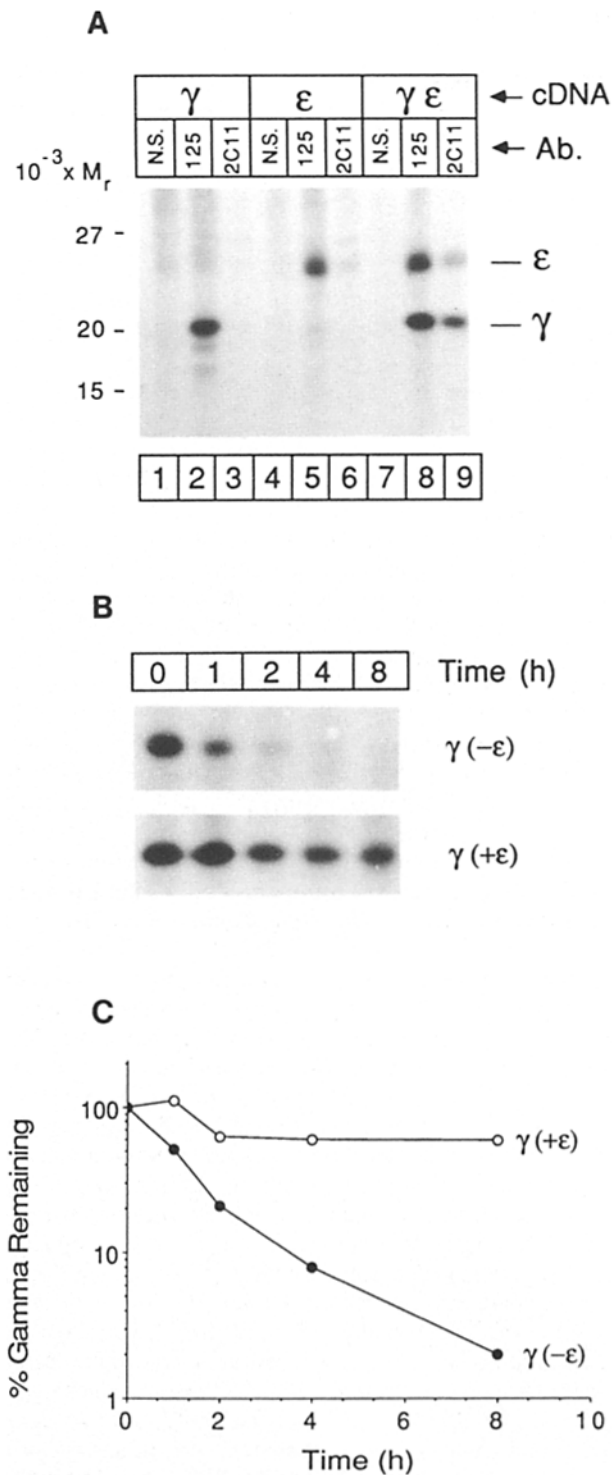
### Multiple Signals for Retention in the ER

Sorting or targeting signals can be defined as structural features within proteins that determine their cellular localization and transport. Signals can occur in the form of prosthetic groups, defined amino acid sequences, or conformational determinants. In its broadest sense, the definition of signal also applies to more variable and less well-defined physicochemical characteristics of proteins (e.g., hydrophobic regions, formation of aggregates). Retention of proteins in the ER has been shown to be mediated by at least two classes of signals. The first class of signals includes linear stretches of amino acids, the best characterized of which is the tetrapeptide KDEL found at the COOH terminus of several resident ER proteins (Munro and Pelham, 1987). This structural motif appears to be responsible for the retention of proteins in the ER (Munro and Pelham, 1987), or in an ER-*cis* Golgi cycling pathway (Pelham, 1988), presumably by binding to a specific receptor. A second less specific mechanism appears to operate for the retention of proteins that lack this signal. A number of studies over the past few years have shown that the conformation of newly synthesized proteins can determine whether they are transported out of the ER or are retained (for review, see Rose and Doms, 1988). The exact nature of the signals involved in this retention mechanism is not known. Two alternative and not mutually exclusive possibilities are that malformed proteins or incompletely assembled complexes either have signals for retention in the ER or lack signals for transport to the Golgi system.

We have shown that incomplete assembly of some TCR complexes results in their retention in the ER. This retention is most probably mediated by the same mechanism that acts on malformed or oligomerization-defective proteins. Irrespective of the actual mechanism, the multiprotein structure of the T-cell antigen receptor provided us with an opportunity to test whether any single subunit possesses the information that determines retention in the ER. The studies reported in this paper, and previous studies, have shown that a number of incomplete structures all possess the information that leads to retention in the ER. Complexes lacking the  $\beta$  (Chen et al., 1988),  $\alpha$  (Furley et al., 1987; Hannum et al., 1987), or  $\delta$  chains (this study) as well as  $\alpha$ ,  $\beta$ , and  $\gamma$  chains expressed separately in fibroblasts (this study; Lippincott-Schwartz et al., 1988) all fail to be transported into the Golgi system. These observations support the idea that information that causes retention in the ER is not the property of a single TCR chain or configuration.

One of the unique observations made with the TCR complex is that not all subunit deficiencies result in retention in the ER. For instance, association of the  $\zeta_2$  homodimer is not required for the efficient transport of TCR complexes into the Golgi system (Minami et al., 1987; Sussman et al., 1988). This observation defines the minimum T-cell receptor complex that to date has been found to be competent for





**Figure 8.** Stabilization of the  $\gamma$  chains by assembly with  $\epsilon$ . (A) COS-1 cells expressing the mouse  $\gamma$  (lanes 1-3) or  $\epsilon$  chains (lanes 4-6), or both  $\gamma$  and  $\epsilon$  (lanes 7-9) were metabolically labeled with [<sup>35</sup>S]methionine for 30 min at 37°C. After solubilization with 0.5% Triton X-100, TCR chains were isolated by immunoprecipitation with either the polyclonal anti- $\gamma\epsilon$  antiserum No. 125 or the monoclonal anti- $\epsilon$  antibody, 2C11. The polyclonal anti- $\zeta$  antiserum No. 123 was used as a control for nonspecific (NS) immunoprecipitation. The positions of molecular weight markers (in 10<sup>-3</sup> × M<sub>r</sub>) appear to the left. The positions of the  $\epsilon$  and  $\gamma$  chains are indicated on the right. (B) After labeling for 30 min at 37°C with [<sup>35</sup>S]methionine, COS-1 cells transfected with  $\gamma$  alone, or  $\gamma$  and  $\epsilon$ , were

efficient transport into the Golgi system:  $\alpha\beta\gamma\delta\epsilon$ . This complex, however, is not efficiently transported to the cell surface but is delivered to lysosomes for degradation (Minami et al., 1987; Sussman et al., 1988).

#### Susceptibility to Pre-Golgi Degradation Is an Inherent Property of Each TCR Subunit

Our previous studies have shown the existence of a non-lysosomal pathway for the degradation of newly synthesized proteins, localized to the ER or a closely related organelle (Lippincott-Schwartz et al., 1988; Chen et al., 1988). In T cells that synthesize all of the TCR chains, complete complexes are assembled in the ER and rapidly transported into the Golgi system. However, in variant T cells that fail to synthesize some of the receptor chains or in fibroblasts that express individual TCR chains, partial complexes or free chains are retained in the ER, where they undergo selective degradation (this study; Chen et al., 1988; Lippincott-Schwartz et al., 1988). Similarly, blocking transport between the ER and the Golgi system in normal T cells using pharmacological agents such as monensin or brefeldin A results in degradation of some of the receptor chains (Lippincott-Schwartz et al., 1988). Comparison of the fates of TCR chains in the various T-cell lines studied has uncovered a common pattern of selective degradation: whereas the  $\epsilon$ ,  $\gamma$ , and  $\zeta$  chains are stable and survive long term (this study; Chen et al., 1988) the  $\alpha$ ,  $\beta$ , and  $\delta$  chains are rapidly and efficiently degraded (this study; Lippincott-Schwartz et al., 1988; Chen et al., 1988). The different fates could be the result of (a) an intrinsic susceptibility of each TCR chain to degradation by the pre-Golgi, nonlysosomal pathway; (b) an effect of subunit interactions on the sensitivity to degradation; or (c) a combination of both factors.

To distinguish between these possibilities, genes encoding individual TCR chains were transfected into COS-1 cells and the fate of individual chains examined. We observed that, even when expressed alone, TCR chains are degraded at very different rates. Thus, it appears that structural features within each chain determine not only retention in the endoplasmic reticulum but also whether they are to be degraded or not. Since we have not yet established the identity of the pre-Golgi degradative organelle, we cannot ascertain whether this differential degradation is a reflection of the quality of TCR chains as substrates for proteolytic enzymes or represents the probability of being sorted to a degradative compartment. In favor of the first possibility, it should be noted that cellular proteases exist that have a relatively restricted specificity. For instance, Ca<sup>2+</sup>-dependent neutral proteases present in human erythrocytes degrade the membrane proteins designated band 2.1 and 4.1 but do not act on other proteins (Pontremoli et al., 1984). This differential sensitivity appears to be due to a requirement for specific sequences that

chased for the times indicated in the figure at 37°C. Gamma chains were isolated by immunoprecipitation with the polyclonal anti- $\gamma\epsilon$  antiserum No. 125 and resolved by SDS-PAGE under reducing conditions. (C) Bands shown in B were scanned by densitometry and the results were plotted as the percentage of the initial  $\gamma$  remaining at each time point. ●,  $\gamma$  alone; ○,  $\gamma$  (+ $\epsilon$ ). Notice the dramatic stabilization of the  $\gamma$  chains produced by co-expression with  $\epsilon$ .

can be cleaved by the enzymes (Melloni et al., 1984). Even the very active lysosomal proteases show some selectivity. For instance, dinitrophenylated serum albumin and anti-dinitrophenyl IgG internalized as a complex via Fc receptors in macrophages were found to be degraded at very different rates upon delivery to lysosomes (Mellman and Plutner, 1984).

### Formation of Stable Oligomers Can Modify the Rate of Degradation

Results from a number of studies have suggested there is greater resistance to intracellular proteolysis displayed by oligomers compared to monomers. For instance, hemoglobin monomers have been shown to be much more efficiently degraded by cytosolic proteases than tetramers (Melloni et al., 1984). Similarly, monomers of the influenza hemagglutinin precursor HAO are more sensitive than trimers to cleavage by several proteases in vitro (Gething et al., 1986; Copeland et al., 1986). These observations suggest that sites sensitive to cleavage by proteases can be masked by the association of protein monomers. Our studies with the TCR indicate that even though each receptor chain has a characteristic sensitivity to pre-Golgi degradation, oligomerization can result in stabilization of the sensitive chains. This is indicated by the stability displayed by the  $\gamma$  chains when they are bound to the intrinsically stable  $\epsilon$  chains (Fig. 7). Not all subunit interactions within the TCR complex result in increased resistance to degradation for the individual chains, however. For instance,  $\delta$  chains in the  $\beta$ -deficient T-cell line 21.2.2 (Chen et al., 1988),  $\alpha$  chains in the  $\delta$ -deficient line  $\Delta 2$  (this study; our unpublished observations), or  $\alpha$  and  $\beta$  chains in normal T cells treated with pharmacological agents that prevent movement out of the ER (Lippincott-Schwartz et al., 1988) are assembled with the stable  $\epsilon\gamma$  core, but are nevertheless efficiently degraded by the nonlysosomal pathway.

What is the explanation for the diverse effect of assembly on the degradation of TCR chains? The topological localization of cleavage sites within the complexes may have an important role. Masking of specific sequences recognized by endoproteases may lead to an increased stability of the assembled subunits. By contrast, if the cleavage sites continue to be exposed in the complexes, assembly will not modify the rate of degradation. An alternative explanation is that  $\alpha$ ,  $\beta$ , and  $\delta$  may be stable as long as they remain within a complex, but may undergo a time-dependent dissociation within the ER if the complex cannot reach the Golgi system. Dissociation from the stable  $\epsilon\gamma$  dimers would then render the free chains susceptible to proteolysis. Whether the enzymes that degrade the sensitive chains coexist in the ER with the stable  $\epsilon\gamma$  dimers or if sorting of the dissociated chains is required for degradation to occur, is not known at the present time. Further studies are being conducted to differentiate between these two possibilities.

We would like to thank Dr. J. Ashwell for his initial assistance in the development of  $\delta$ -deficient T cells, Drs. S. Hedrick, D. Gold, and M. Crumpton for their gifts of plasmids containing TCR cDNAs, Drs. D. Louvard, J. Bluestone, and J. Allison for their generous gift of some of the antibodies used in this study and Sandoz Company for their gift of brefeldin A. We also thank Drs. L. Samelson and J. Ashwell for critical reviews of the manuscript, and K. Perry and Y. Yi for expert secretarial assistance.

Received for publication 12 December 1988 and in revised form 29 March 1989.

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