Rapid Degradation of an Unassembled Immunoglobulin Light Chain Is Mediated by a Serine Protease and Occurs in a Pre-Golgi Compartment*

(Received for publication, June 18, 1993, and in revised form, August 25, 1993)

Anne M. Gardner, Sigal Avieli, and Yair Argon†
From the Department of Immunology, Duke University Medical Center, Durham, North Carolina 27710

The immunoglobulin κ light chain produced by the CH12 lymphoma is unusual because it is not secreted when expressed in the absence of a heavy chain. Instead, it undergoes rapid intracellular degradation. This degradation is selective, as another light chain expressed in the same cell is not degraded. It is also a property of the CH12 κ chain itself, since it is degraded rapidly when expressed either in another myeloma cell or in COS-1 fibroblasts. When provided a heavy chain, this κ chain assembles into IgM and is then protected from proteolysis. The degradation of κ requires ATP, is sensitive to reduced temperature and to the thiol reagent diamide. Of all the proteolytic inhibitors tested, 3,4-dichloroisocoumarin, L-1-tosylamido-2-phenylethyl chloromethyl ketone, and to a lesser extent 1-chloro-3-tosylamido-7-aminoo-2-heptanone, inhibit κ degradation, suggesting the involvement of a serine protease. The degradation of κ does not require transport to the Golgi complex, nor is it sensitive to a variety of cystosomotropic agents. Both immunofluorescence and the observed association with the endoplasmic reticulum (ER) stress proteins GRP78/BiP and GRP94 indicate that the κ chain is localized mostly in the ER. When a point mutation which blocks transport to the Golgi complex is introduced into this κ chain, the association with the stress proteins is enhanced but the rate of degradation is not significantly decreased. We conclude that the CH12 κ chain is a particularly good substrate for an ER degradation machinery, and that its sensitivity to the protease(s) is governed by its state of assembly. This ER degradation provides a possible quality control mechanism during the differentiation of B lymphocytes.

A characteristic of B lymphocyte differentiation is the nonsynchronous expression of the two immunoglobulin (Ig) subunits. Because gene segments are rearranged sequentially, the μ1 heavy (H) chain is first expressed at the pre-B cell stage without its partner light (L) chain. The fate of this unassembled μ chain is controlled at several levels. Different carboxyl-termini lead to a membrane-bound form and a secreted form. Each of these forms interacts with other polypeptides, essential for further transport of the μ chain. The membrane μ form interacts with Igβ and Igγ proteins (1) and both forms are complexed with surrogate L chains at early developmental stages (2). Much of the newly synthesized μ is degraded intracellularly, and specific structural features of the μ chain itself are known to control this process (3–5). The L chain of Ig also exists in an unassembled form during normal B cell differentiation; plasma cells produce an excess of L chains, which are usually secreted without assembly with other polypeptides (6). A number of variant plasmacytoma lines have been isolated in which expression of H chain has been lost, but L chain expression persists. A few of these variants, such as the λII chain producer MOPC315.37 (7) and the κ chain producer NSI (8), fail to secrete their L chains. In the latter case, the L chains are also subjected to rapid intracellular degradation, but very little is known about the location or biochemistry of their degradation.

Since the catabolism of unassembled Ig subunits is of physiological relevance, we studied their intracellular degradation in a model system, the murine lymphoma CH12 and its derivative CH12k. The latter has lost expression of the μ heavy chain because of a chromosomal deletion encompassing the μ gene (9). CH12k continues to produce a κ light chain, but it is not secreted (9). The CH12 κ chain is encoded by a germline gene (10, 11), and the sequence reveals no unusual substitutions which could explain the failure to be secreted. As we show, this otherwise normal L chain is degraded rapidly, apparently by an ER proteolytic machinery.

A pre-Golgi degradation process, tentatively assigned to the ER (12), has been characterized in recent years through work on subunits of the T cell antigen receptor (13), asialoglycoprotein receptor (14), and HMG-CoA reductase (15). Subunits of all these membrane-spanning proteins appear to be degraded by a common process (reviewed in Ref. 12). A major issue with respect to this pre-Golgi degradation is its specificity. The signals that target HMG-CoA reductase and T cell receptor subunits for degradation are encoded by their trans-membrane segments (15, 16), but in the case of asialoglycoprotein receptor, an exoplasmic, luminal peptide confers sensitivity to degradation (17). Furthermore, one soluble protein, the PiZ mutant of α1-antitrypsin, has been shown to be a substrate for pre-Golgi degradation (18). A second unresolved question is the location of the degradative machinery. The degraded polypeptides bear immature oligosaccharides and their degradation is not affected by inhibition of intracellular transport, nor is it sensitive...
to disruption of lysosomal functions (reviewed in Ref. 12). Studies using permeabilized cells show that the degradation continues under conditions where vesicular movement of protein out of the ER is blocked (19, 20). On the other hand, engineering a KDEL ER retrieval signal on a mutant of α1-antitrypsin blocked its degradation (18). This finding is consistent with inhibition of an obligatory transport step from the ER to a degradative compartment, but is also consistent with physical protection of the substrate by the binding of the KDEL receptor while still in the ER. Thus, it is not still clear whether degradation occurs in the ER per se.

We show here that an Ig κ chain, another soluble protein, is degraded by a process which is selective for this particular κ chain, and is limited to the unassembled state. Furthermore, we provide microscopic, pharmacological, and genetic evidence in support of the ER localization of this degradation and impli-
cate the involvement of a serine protease.

**EXPERIMENTAL PROCEDURES**

**Cell Culture**—The in vitro CH12 lymphoma line was passaged as a transplantable ascites in B10.A mice, as described in Ref. 10. Mice were obtained from the Frederick Cancer Research Facility (Frederick, MD) or the Jackson Laboratory (Bar Harbor, ME). The *in vitro* culture conditions, in endotoxin-free medium, for CH12 cells were as described in Ref. 10. Two *in vitro* adapted variants of the CH12 cell line, CH12LX and CH12K, (formerly CH12LX 2B4 (9), were cultured in endotoxin-free Dulbecco's modified Eagle's medium (DMEM), supplemented with fetal calf serum (JRI Biosciences, Lenexa, KS), glutamine, penicillin, and streptomycin (Gibco) and 15 μM 2-mercaptoethanol (Eastman Fine Chemistries, Rochester, NY). To initiate *in vitro* differentiation of CH12, CH12LX, or CH12K, the B cell mitogen lipopolysaccharide from *Escherichia coli* B (55:56; Difco) was included in the medium at a final concentration of 50 μg/ml (10).

The NS0 myeloma and COS-1 fibroblasts (both from the ATCC, Rock-
ville, MD) were cultured in the same medium without 2-mercaptoeth-
ol. Variants of the CH12LX and NS0 cell lines which express drug
resistance markers were grown in the same medium supplemented with either 2 mg/ml G418 for neo expressing lines (21), or 6.25 μg/ml mycophenolic acid, 250 μg/ml xanthine, 0.1 μM thymidine (22) for lines expressing the bacterial xanthine-guanine phosphoribosyltransferase (*gpt*) gene. Hybrids generated by fusion of these drug-resistant lines were cultured in the presence of both G418 and mycophenolic acid. Under these conditions, drug-sensitive cells died in 2–4 days and resis-
tant lines grew out in 2–3 weeks.

**Stable and Transient Transfections**—κ, μ, or γ chains were expressed in CH12 cells using the vectors encoding the variable and constant region exons of the original and mutated CH12 κ genes were performed with the DEAE-dextran method as described (20). Ig gene expression was ana-
yzed 40–50 h later by immunofluorescence and metabolic labeling.

**Metabolic Labeling, Drug Treatments, and Cell Lysis**—Metabolic labeling experiments were performed as in Refs. 10 and 23 with 75–300 μCi/ml l-[35S]methionine (Trans2S-label, specific activity >1000 Ci/mmol, ICN). When used, drugs and inhibitors were included in the chase medium, except where pretreatment is indicated. After cells and supernatants were separated by centrifugation, the cell pellet was lysed with ice-cold Nonidet P-40 lysis buffer (25). In some experiments the lysates were cross-linked with dithiothreitol (sucinimidyl propionate) (DSP) (25).

**Immunoprecipitation and Electrophoresis**—Ig chains were isolated by immunoadsorption with rabbit anti-κ serum, nonspecific rabbit anti-λ serum, or rabbit anti-μ serum, followed by protein A-Sepharose (Pharmacia LKB Biotechnology Inc., Piscataway, NJ), or by adsorption onto SM1/45-Sepharose. SM1/45 is a monovalent rat IgG1 anti-mouse μ-chain (25). The Ig-binding protein, BiP, was immunoprecipitated using rat anti-BiP monoclonal (27) and monoclonal anti-rat-μ (RG-7) (28) coupled to Sepharose beads. The labeled proteins were resolved by electrophoresis on 10% SDS gels (29) followed by fluorography (30). Autoradiographs were quantified by densitometry using a Hewlett–Packard scanner and Image 1.29 software (NIH).

**Immunofluorescence**—Lymphoid cells were allowed to attach to poly-
L-lysine coated slides, while transfected COS cells were grown on coverslips. Cells were fixed for 1 h in fresh 2% paraformaldehyde, 0.1% glutaraldehyde in PBS at room temperature. The fixative was quenched with three 15-min washes in 50 mM NH4Cl in PBS, and the cells were permeabilized with 1% Nonidet P-40, 0.01% saponin, 0.25% gelatin, PBS for 10 min at room temperature. After removal of the permeabi-
linizing solution, the cells were incubated with antibodies diluted in
0.1% saponin, 0.25% gelatin, PBS. For double-label immunofluores-
cesence, the cells were incubated simultaneously with rabbit anti-mouse κ serum plus either monoclonal rat anti-BiP antibody or wheat germ agglutinin-biotin. After a 1-h incubation at room temperature, the cells were washed 6 times and incubated with fluorescent secondary anti-
odies or streptavidin for an additional hour. Following 6 washes, the cells were mounted in 0.25% 1,4-diazabicyclo(2,2,2)octane (Polysciences), 90% glycerol in PBS and examined with a Zeiss axiophot micro-
scope equipped with a laser scanning confocal head (Bio-Rad MRC
600 system), using narrow band width filters for fluorescein and Texas-
Red. Photographs were taken using T-MAX 100 film (Kodak, Rochester, NY).

**ATP Assays**—ATP levels were measured using a luciferase assay kit from Sigma, according to the manufacturer's instructions, with a Ber-
thold LB 9500C luminometer. Relative ATP levels were calculated by
comparing the signal from untreated cells with the signal from cells
treated with various concentrations of artemisin A. Duplicate cell ly-
sates were prepared for each treatment condition, and ATP determina-
tions were performed in triplicate. Standard deviations were less than
10%.

**Chemicals and Reagents**—Chemicals were obtained from Sigma, un-
less stated otherwise. DNA restriction and modifying enzymes, as well as endoglycosidase H, tunicamycin, and 3,4-dichloroisocoumarin (3,4-
DCI) were from Boehringer Mannheim. Brefeldin A was a generous gift of Dr. R. Yewdell (NIH).

**RESULTS**

**Free CH12 κ Chains Are Not Secreted**—Radiimmunoassay to assess L chain secretion by the CH12κ variant indicated that L chain was produced, but not secreted (9). To confirm this result, we compared Ig κ chain synthesis and secretion in the CH12 and CH12κ cell lines by pulse-chase analysis and immu-
noprecipitation. CH12 κ cells synthesized a κ chain which rapidly
associated with the μ chain during the 15-min pulse period, and

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the assembled IgM was secreted with a half-time of approximately 1 h (Fig. 1A and Ref. 31). The increased apparent molecular mass of the secreted μ chains reflected the processing of oligosaccharide side chains during transit through the Golgi complex. In contrast to CH12 cells, the vast majority of free κ chain made by CH12κ cells was not secreted (Fig. 1B). Only a small amount of κ was detected in the medium even after 3 h, and densitometry of the autoradiographs showed that this secreted fraction was less than 5% of the total κ synthesized.

The secretory failure of CH12κ is a property of the L chain itself; it was not secreted even when expressed in the myeloma variant NS0 or in COS-1 fibroblasts (data not shown). Furthermore, the secretory failure occurs only when the κ chain is not assembled. In the CH12κμ transfectant, which expresses endogenous κ and μ chains assemble with all these μ chains and are secreted efficiently as part of IgM molecules (Fig. 2).

**Free CH12 κ Chains Are Degraded Rapidly**—As can be inferred from Fig. 1B, the free κ chains were degraded intracellularly. To determine the initial rate of degradation, CH12κ cells were pulse-labeled with [35S]methionine for 5 min and chased for up to 180 min. As shown in Fig. 3, degradation commenced immediately, without an appreciable lag, and the half-life of κ was estimated to be 40 ± 10 min. This degradation rate is remarkably rapid as compared to the turnover of many other proteins. When expressed in COS-1 cells, the half-life of the CH12 κ was also short, 60 ± 15 min, whereas the half-life of many other L chains in COS cells is 100 min or longer (Ref. 23 and see Table II). Thus, a free CH12 κ chain is a substrate for accelerated intracellular degradation.

**The Degradation Is Selective**—By expressing a second L chain in the CH12κ line we were able to show that the degradation of the CH12 κ chain is selective. The L chain used was a chimeric molecule encoded by a κ gene variable region linked to a μ constant region (VμCHY87CA (23)). This light chain was chosen because it is secreted efficiently (23) and can be distinguished from the endogenous κ chain by both immunoreactivity with anti-μ serum and by its reduced gel mobility. Several clones expressing the chimeric L chain were isolated and analyzed by pulse-chase experiments. As can be seen in Fig. 4, the chimeric L chain was not degraded; its recovery in the culture medium after 3 h of chase exceeded 85% of the amount synthesized during the pulse. The CH12 κ chain was still degraded selectively in all the CH12κα clones analyzed. We conclude that some feature of the sequence of the CH12 κ chain targets it for rapid degradation.

**Degradation Does Not Require Transport Through the Golgi Complex and Is Nonlysosomal**—To determine if passage through the Golgi complex is needed for the intracellular degradation of κ, we examined the effect on degradation of inhibitors of intracellular transport. As shown in Table I, treatment of cells with monensin, an ionophore which interferes with intra-Golgi transport (32), had no effect on κ degradation. Likewise, no inhibition of κ degradation was observed after treatments with either carbonylcyanide m-chlorophenylhydrazone or brefeldin A (Table I), two agents which block transport from the ER to the Golgi complex by different mechanisms (25, 33, 34). Thus, transport to or through the Golgi complex is not required for κ proteolysis.

Another possible mechanism for κ degradation is direct transport from the ER to lysosomes via autophagic vesicles.
Effect of various inhibitors of intracellular transport, metabolism, and lysosomal action on \( \kappa \) degradation

| Treatment | Dose | \( \kappa \) remaining |
|-----------|------|----------------------|
| None      |      | 38                   |
| Monensin  | 10 mM| 47                   |
| CCCP      | 10 mM| 43                   |
| Brefeldin A | 2 \( \mu \)g/ml | 37                   |
| Brefeldin A | 10 \( \mu \)g/ml | 42                   |
| \( \text{NH}_4\text{Cl} \) | 30 mM| 37                   |
| Chloroquine | 100 mM| 44                   |
| Leupeptin  | 100 mM| 35                  |
| Chymostatin| 100 mM| 37                  |
| None      |      | 32                   |
| Diamide   | 0.1 mM| 40                   |
| Diamide   | 1 mM | 41                   |
| 37 °C     |      | 32                   |
| 24 °C     |      | 96                   |
| 19.5 °C   |      | 97                   |
| 4 °C      |      | 95                   |

*CH12x cells were labeled for 30 min with \( ^{35} \text{S} \)methionine and chased for 1 h. They were incubated with the indicated drugs, or at the indicated temperatures, during the chase only. The immunoprecipitated \( \kappa \) bands were quantified by densitometry of the gels.

**Monesinin was dissolved in ethanol, carbonyl cyanide \( m \)-chlorophenylhydrazone (CCCP) and brefeldin A in dimethyl sulfoxide, and the other inhibitors in water.

* Present during 1-h pretreatment as well as during the pulse and chase.

To address this possibility, we tested the effects of lysosomotropic agents on the half-life of \( \kappa \). As shown in Table I, degradation of \( \kappa \) was not affected by treatment of the cells with weak bases (\( \text{NH}_4\text{Cl} \) or chloroquine), which raise intravesicular pH (36). Inhibitors of endosomal and lysosomal proteases (leupeptin and chymostatin) (37) also showed no effect on \( \kappa \) degradation (Table I). These results argue against lysosomal and autophagic involvement in the degradation of \( \kappa \), and support a pre-Golgi site of degradation.

\( \kappa \) Chain Degradation Is Sensitive to the Redox State of the Cell—One drug which did abolish the degradation of \( \kappa \) was diamide. Treatment of CH12x cells with this agent, which oxidizes glutathione (38), inhibited degradation (Table I). In the presence of 1 mM diamide, only 9% of the \( \kappa \) chain was degraded in 1 h, in marked contrast to untreated control cells. Therefore, a reducing environment, which is the normal condition in the lumen of the ER (39), may be necessary for the degradative process.

A cysteine residue in the secretory tail of \( \mu \) chains provides a signal for ER retention and selective degradation of these chains, and treatment of whole cells with \( \beta \)-mercaptoethanol overcomes this effect (40). Therefore, we tested the effect of \( \beta \)-mercaptoethanol on the degradation of CH12 \( \kappa \). However, treatment of CH12x cells with up to 14 mM \( \beta \)-mercaptoethanol had no effect on \( \kappa \) degradation or on its secretion (data not shown). Hence, the mechanism of L chain degradation differs from that of \( \mu \) chains.

Low Temperature Inhibits \( \kappa \) Chain Degradation—Temperature dependence of \( \kappa \) degradation was studied by chasing \( ^{35} \text{S} \)methionine-labeled CH12x cells at 37, 24, 19.5, or 4 °C. As shown in Table I, incubation at temperatures of 24 °C or lower effectively blocked the degradation: less than 6% of the pulse-labeled \( \kappa \) chain was degraded in 1 h. Thus, the degradative process involves either temperature-sensitive transport to a proteolytic compartment or a temperature-sensitive component of the proteolytic machinery itself.

Intracellular \( \kappa \) Chains Are Localized Largely to the ER—The intracellular distribution of \( \kappa \) chains in CH12x and in the parental CH12 line was examined by immunofluorescence and confocal microscopy. A series of confocal sections through CH12x cells stained with anti-\( \kappa \) serum revealed a network of fine reticular structures throughout the cytoplasm (see Fig. 5A for one such optical section), as well as prominent staining of the nuclear envelope. The optical sections showed no perinuclear staining, and the cell surface was negative. This fluorescence pattern is characteristic of the ER. In contrast, CH12 cells exhibited strong perinuclear staining, as well as cell surface fluorescence (not shown), as expected for a cell which transports assembled IgM to the plasma membrane.

The distribution of \( \kappa \) was assessed more precisely by double-label immunofluorescence with markers for the ER and the Golgi complex. As shown in Fig. 5, B-C, \( \kappa \) co-localized quite precisely with BiP, a resident ER protein (27). Both proteins were present in the nuclear envelope and in identical reticular structures throughout the cytoplasm. At this level of resolution, there was no evidence for restricted distribution of \( \kappa \) chain in domains within the ER. The Golgi complex was identified in CH12x cells with wheat germ agglutinin, which binds to the complex oligosaccharides formed in this organelle (41). Double-label immunofluorescence showed the \( \kappa \)-positive structures to be distinct from wheat germ agglutinin-positive structures, indicating the paucity of \( \kappa \) in the Golgi complex (Fig. 5, panels D and E). Immunofluorescence of COS-1 cells expressing the CH12 \( \kappa \) chain, where the ER can be distinguished at higher resolution than in B cells, also showed that most of \( \kappa \) chain is in the ER (data not shown).

The Intracellular \( \kappa \) Chain Is Associated with Two ER Stress Proteins—Independent evidence that a large population of the \( \kappa \) chains resides in the ER was obtained by identifying the proteins associated with \( \kappa \). CH12x cells were labeled metabolically with \( ^{35} \text{S} \)methionine for 3.5 h and then lysed in 0.2% non-dissolving detergent. CH12x cells were fixed, permeabilized, and incubated: A, with rabbit anti-\( \kappa \) serum, followed by fluorescein isothiocyanate-conjugated goat anti-rabbit IgG; B and C, with both rabbit anti-\( \kappa \) serum and rat anti-BiP monoclonal, followed by fluorescein isothiocyanate-conjugated goat anti-rabbit IgG and Texas red-conjugated streptavidin. Panels A, B, and D show \( \kappa \) fluorescence. Panel C shows staining of BiP, and panel E shows wheat germ agglutinin staining of the fields in B and D, respectively. Scale bars, 10 \( \mu \)m.
digitonin in the presence of DSP, a thiol-cleavable cross-linker (42), in order to trap weak interactions with other proteins. We used this method previously to examine proteins associated intracellularly with Ig chains (26). Cross-linked and noncross-linked cell lysates were immunoprecipitated with anti-κ serum and approximately equal numbers of immunoprecipitable κ counts were analyzed by SDS-PAGE. As shown in Fig. 6, lanes 1–2, two major proteins specifically co-precipitated with κ chains. One protein of molecular mass 78 kDa was present in both cross-linked and noncross-linked lysates. This protein has been identified as BiP, since it co-migrated with immunoprecipitated BiP (Fig. 6, and see Ref. 26), and is recognized by monoclonal anti-BiP antibody in immunoblots (26, 27). Another band migrating slightly faster than BiP has been detected frequently (see Fig. 6). It is not presently known whether it is a different polypeptide or a modified form of BiP. A third polypeptide which associated with CH12 κ was enriched by the DSP cross-linking. This polypeptide had been identified as the ER stress protein GRP94/ERP99 (26). This pattern of associated proteins was also seen when the CH12 κ chain was expressed in the myeloma NS0 (Fig. 6, lanes 3 and 4) or in COS cells (not shown). Since both BiP and GRP94/ERP99 are resident ER proteins, these results reinforce the microscopy data in pointing to the ER as the subcellular site of the κ chain.

CH12 κ Is Not Protected from Degradation by a Mutation Which Blocks Transport to the Golgi Complex and Enhances BiP and GRP94 Binding—Because of the limitations inherent to immunofluorescence and to the pharmacological approaches, we sought another criterion to determine the site of κ degradation. To this end we introduced into the CH12 κ gene a point mutation, substituting its Arg51 with Thr. As shown elsewhere, this mutation causes transport arrest of L chains in the ER. When introduced into an 4-hydroxy-3-iodo-5-nitro-phenylacetic acid-specific Al L chain, this mutation is sufficient to retain this L chain in the ER as judged both by double-label immunofluorescence and by the increased association with both BiP and GRP94. The RT61 mutation has no measurable effect on the folding of the L chain or on its ability to assemble with H chains into functional, 4-hydroxy-3-iodo-5-nitro-phenylacetic acid-binding Ig. These Ig molecules are arrested in an endo H-sensitive stage and are not secreted. When RT61-mutated CH12 κ chains were expressed in COS cells, their transport was completely arrested and even the residual secretion seen with CH12 κ was abolished. As judged by co-immunoprecipitation, 3-fold more BiP and 6-fold GRP94 associated with CH12 κRT61 than with the original chain (Table II). Despite this higher association, CH12 κRT61 is still degraded rapidly, at a rate similar to that of CH12 κ (Table II). This experiment provides further evidence that transfer out of the ER is not required for κ turnover, and that degradation actually occurs in the ER itself. A second interesting conclusion is that the association with two ER stress proteins does not protect this κ chain from degradation.

Depletion of Cellular ATP Inhibits κ Degradation—The degradation of most proteins in eukaryotic cells requires metabolic energy (43). The role of ATP in κ degradation was investigated by depleting cellular ATP with antimycin A (44). In parallel samples ATP levels and the amount of κ left after 1 h of treatment with various concentrations of antimycin A were measured. The amount of κ chain present was inversely proportional to the level of ATP (Fig. 7A), indicating that the degradation requires ATP. ATP depletion by antimycin A was rapid: within 5 min after the addition of antimycin A, ATP pools were depleted, and there was no further loss over the next hour (Fig. 7B). In pulse-chase studies, antimycin A at 100 μM substantially increased the half-life of κ. Fig. 7C shows that all of the newly synthesized κ chain remained after 1 h of chase in the presence of antimycin A, while only 33% remained in untreated samples. The inhibition of κ degradation was still apparent after 2 h of treatment. Furthermore, the effect of antimycin A was reversible; removal of the inhibitor and addition of fresh medium containing glucose rapidly increased the amount of ATP, with concomitant resumption of κ degradation (data not shown).

Since ATP depletion also blocks protein synthesis, we investigated whether κ degradation requires new protein synthesis. Cycloheximide had no effect on κ degradation at 100 μg/ml, a level which inhibited 98% of protein synthesis. Thus, κ degradation does not require ongoing protein synthesis. Together with the data suggesting that transport out of the ER is not required, the effect of antimycin A indicates that the proteolysis itself is ATP-dependent.

κ Chain Degradation Is Inhibited by Serine Protease Inhibitors—To determine the specificity of the degradation, several types of membrane-permeable protease inhibitors were tested. The most effective of them was the mechanism-based serine protease inhibitor 3,4-DCI (45). A time course of κ chain degradation in the presence of 3,4-DCI (Fig. 8) showed that 98% of newly synthesized κ chains were protected from degradation.

| L chain   | BIP/L chain ratio | GRP94/L chain ratio | Half-life (min) |
|-----------|------------------|---------------------|-----------------|
| CH12 κ    | 0.29             | 0.07                | 60 ± 15         |
| CH12 κRT61| 0.83             | 0.42                | 45              |
| AIRT61    | 0.66             | N.D.                | 100             |

a COS cells expressing the indicated L chain were labeled for 30 min with [35S]methionine and lysed in the presence of DSP. After immunoprecipitation with anti-κ or anti-λ antibodies and SDS-PAGE, the amounts of labeled L chain and co-precipitated BiP bands were quantified by densitometry of the gels and the ratio of BiP to L chain within each sample was calculated.

b Determined from the same experiments as above. Note that the labeling of GRP94 under these conditions is rather poor, probably due to the long half-life of GRP94, so its quantitation is less reliable.

c Determined by densitometry of autoradiograms of pulse-chase experiments like the one described in Fig. 3.

d This Thr-for-Arg substitution arrests the transport of Al light chains in the ER.
Selective degradation is emerging as an important control mechanism used throughout the B cell lineage to regulate the fate of Ig chains. In pre-B cells, which produce both the membrane and secreted forms of μ chain, the membrane form is selectively degraded (5). In mature B cells, the converse situation exists, selective degradation of the secreted form of Ig and transport of membrane Ig to the cell surface (46). Plasma cells often synthesize an excess of L chains, which if not secreted are disposed of by intracellular degradation (8). In all these cases the selective intracellular degradation is non-lysosomal, but its mechanism and the compartment where it occurs are as yet poorly characterized (12). In this work we characterized the degradation of an unassembled Ig κ chain and show it to occur prior to arrival at the Golgi complex, most likely in the ER itself.

The CH12 cell is representative of the transition from B cells to plasma cells, the so-called "pre-plasma cell" stage (47), and is already primed for increased synthesis of Ig. Importantly, its κ chain is subjected to intracellular degradation although it is encoded by a germ-line gene that has not undergone somatic mutation (10, 11). In fact, this L chain is perfectly functional, as shown by its assembly with a transfected H chain to form anti-erythrocyte IgM. Thus, the CH12 κ chain is targeted for degradation only in the absence of subunit assembly, much like the case of the unassembled T cell receptor components (13).

Most of the characterization of pre-Golgi degradation of exported proteins comes from studies on membrane proteins such as the T cell receptor, asialoglycoprotein receptor and HMG-CoA reductase (14, 16, 48-51). These studies outlined properties of the pre-Golgi degradation process which distinguish it from lysosomal degradation. One soluble protein, a non-secreted mutant α1-antitrypsin, was also shown to be degraded without transport to the Golgi complex (18, 52). The κ chain of CH12 is a second example of a degraded soluble protein, but unlike the α1-antitrypsin mutant it is a normal, functional polypeptide. As we report here, many of the biochemical properties of the degradation of membrane-bound polypeptides are also shared by soluble, luminal proteins.

As we show, the degradation of CH12 κ is dependent on ATP, temperature, and the redox potential. One property which is unique to the degradation of CH12 κ is that it commences without any obvious lag time after synthesis. In contrast, the degradation of other substrates begins after a clear lag of 20-30 min (15, 48, 49), and in one case (18) this lag is pH dependent. Although the significance of this observation is not yet known, the lack of a lag period emphasizes that this κ chain is targeted for degradation very soon after synthesis. In any case, the similar properties of degradation of soluble secreted proteins and plasma membrane proteins strongly suggest that the same enzymatic machinery is involved.

We further show that the degradative enzyme has the inhibitor spectrum of a serine protease. A serine protease is also
implicated in the degradation of the asialoglycoprotein receptor H2 chain (51). On the other hand, the degradation of T cell receptor chains may be mediated by a cysteine protease (53). Relatively little is currently known about ER proteases: egasyn has been identified as an esterase in the ER of kidney cells (54), and ER-60 has been identified as a cysteine protease of the ER (55). In yeast, an experimentally modified form of the Kex2 protease (which usually resides in a Golgi subcompartment) can function as a luminal ER protein, degrading passenger proteins (56). It is likely that other proteases exist as resident ER proteins, and our current experiments are designed to identify them.

Since the CH12 κ chain is degraded while another L chain in the same compartment is unaffected, the enzymatic machinery involved is very selective. There must be specific signals which mark such polypeptides as appropriate targets. The signals which target HMG-CoA reductase and T cell receptor α chain for degradation have been shown to reside in the trans-membrane regions of these proteins (15, 16, 57). On the other hand, in the case of the H2 subunit of the asialoglycoprotein receptor the signal for degradation seems to reside in the luminal portion adjacent to the trans-membrane region (17). The signals to recognize Ig L chains are likely to be different yet. Because most κ chains, bearing an identical constant domain to CH12 κ, are not subjected to fast degradation, we surmise that the signal for CH12 κ degradation is in its variable domain. The availability of a large family of Ig L chains should facilitate the identification of structural features which signal degradation.

An important question about the pre-Golgi degradation of κ is its precise location: does it occur in the ER itself or in another compartment? In addition to the co-localization of κ chains with BiP by microscopy, we show that κ degradation begins within minutes of synthesis and that its rate is insensitive to drugs that block egress from the ER. Furthermore, we show that a mutation which limits the κ chain to the ER also fails to protect it from rapid degradation. Although these data provide negative evidence, taken together they suggest the ER itself as the site of degradation. This is in agreement with data about the site of the first cleavage of the H2 subunit of asialoglycoprotein receptor (51).

Like other nonsecreted proteins, CH12 κ chains are found in association with the luminal ER protein BiP. It has been proposed that BiP dissociates from substrates prior to their degradation (58), but its role in targeting polypeptides for degradation or in protecting them has remained unclear. The amount of BiP bound to CH12 κ is only about half of what is found associated with other ER-arrested mutant L chains (Refs. 26 and 59 and Table II). Importantly, increasing the binding of BiP to the CH12 κ chain (by incorporating the RT61 substitution) did not increase its stability, demonstrating that BiP has no protective role. In addition, BiP does not appear to target the L chain for degradation, since a λ chain containing the equivalent RT61 mutation and which binds BiP to the same extent is much more stable than the CH12 κRT61 mutant. This result is consistent with the observation that the P2Z mutant of α1-antitrypsin is subject to pre-Golgi degradation without any apparent association with BiP (18). Therefore, BiP binding seems to reflect the extent of altered folding of the mutants and not their targeting for degradation.

In addition to BiP, GRP94/ERp99 is specifically associated with the intracellular κ chains. Although the physiological role of GRP94 is unknown, its association with intracellular L chains and BiP suggests that it too is involved in folding and assembly (26), as has been suggested for BiP (60). BiP and GRP94 were found associated with chains like CH12 κ which are degraded rapidly, as well as with more stable L chains (26). This argues against a direct role for either stress protein in the pre-Golgi degradation pathway.

It is interesting to compare the characteristics of κ degradation with those described for the degradation of assembled secretory IgM (61). Together, the data indicate that free subunits and assembled IgM may be degraded in different compartments. Amitay et al. (61) showed that the degradation of assembled secretory IgM occurs not in the ER per se, but in a more distal compartment intermediate between the ER and the Golgi complex. Unlike IgM degradation, κ degradation is not inhibited by brefeldin A treatment. Therefore, it seems likely that the degradation of free L chains is executed in the ER while the degradation of secretory IgM occurs in an intermediate compartment. Moreover, degradation of secretory IgM was best inhibited by a cysteine protease inhibitor while the best inhibitors of κ degradation were serine protease inhibitors. It is possible that whereas the selective degradation of IgM in B cells is under developmental control and may be tissue specific, the degradation of free L chains occurs in B cells, plasma cells, and even fibroblasts by a ubiquitous ER machinery.

In the later stages of the B cell lineage, as large-scale production of Ig begins, the presence of nonsecretable Ig chains in the ER at high concentrations may lead to the formation of Russell bodies (62), and these inclusion bodies can be pathogenic (63, 64). Thus, an efficient degradative process to dispose of the nonsecreted proteins in the ER would be an important quality control measure to ensure the survival of the cell.

Acknowledgments—We thank Drs. L. Arnold and G. Haughton (University of North Carolina) for providing the CH12 cell line and its derivatives. We also thank Dr. M. Ostrowski (Dept. of Microbiology, Rockefeller Medical Center) for the use of his immunofluorometer, Dr. F. Schachat (Dept. of Cell Biology) for the use of his scanner, Dr. R. Corley (Dept. of Immunology) for providing the genomic CH12 κ clone, and Dr. J. Yewdell (NIH) for a gift of brefeldin A. Finally, we thank D. Wiest, J. Burkhardt, J. Meinick, and J. Dul for comments on the manuscript and suggestions throughout this work.

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* A. M. Gardner, S. Aviel, and Y. Argon, unpublished results.
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