Distinct Intracellular Fates of Membrane and Secretory Immunoglobulin Heavy Chains in a Pre-B Cell Line

Anand K. Bachhawat and Shiv Pillai

Molecular Immunology Laboratory, Molecular Genetics Group, The Cancer Center of Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts 02129

Abstract. The intracellular fates of membrane and secretory immunoglobulin heavy chains were examined in a pre-B cell line that has switched to the γ isotype. The membrane form of the heavy chain (γm) was rapidly degraded while the secretory form (γs) was retained intracellularly in association with BiP. The degradation of γm could not be inhibited by ammonium chloride, chloroquine, or monensin suggesting that it occurred in a nonlysosomal compartment. The inability to detect any Endo H–resistant form of γm before its degradation suggested that degradation occurs before entry into the Golgi compartment. Degradation of γm could be inhibited by incubation at 24°C. In a derivative of this cell line expressing a transfected κ gene, γs formed disulfide linked tetramers with κ and was secreted, while γm, although associated with κ, continued to be rapidly degraded. These observations suggest that membrane and secretory heavy chain proteins are retained by distinct intracellular mechanisms. Although masking of the CH1 domain abrogates γs retention, this domain does not influence the intracellular fate of γm.

THE transport of cell surface as well as secretory proteins depends on proper folding and the complete assembly of oligomers (Kreis and Lodish, 1986; Gething et al., 1986; Hurtley and Helnius 1989). Misfolded proteins and incomplete oligomers are retained intracellularly; although retention signals have been identified for some proteins, the mechanisms involved in the process of retention remain poorly understood.

The expression of membrane and secretory immunoglobulins is regulated during B cell ontogeny not only at the levels of transcription and posttranscriptional processing but also at the level of intracellular transport. These proteins offer a unique opportunity for the analysis of the signals and mechanisms that influence the retention and transport of nonanchored secretory proteins in comparison with their membrane-anchored counterparts.

Two retention signals have been identified in incompletely assembled secretory immunoglobulins. One of these signals is the CH1 domain of all immunoglobulin heavy chains, which unless masked by association with a light chain leads to retention. These retained heavy chains are associated with the immunoglobulin heavy chain binding protein (BiP; GRP 78) (Morrison and Scharff, 1976; Haas and Wabl, 1983; Munro and Pelham, 1986). BiP is an ER protein that is itself retained within the ER by virtue of a KDEL sequence at its carboxyl terminus (Pelham 1988) and presumably binds the CH1 domain of immunoglobulin heavy chains (Bole et al., 1986; Hendershot et al., 1987; Hendershot, 1990). In cells that lack light chains, deletions in the CH1 domain lead to secretion of the heavy chain γ isotype. In the case of secretory μ and α heavy chains however, in addition to the CH1 retention signal, a second retention signal is present in the cysteine containing tailpiece in the CH4 domain. μs molecules in which the CH1 domain has been deleted are still retained intracellularly and bind BiP (Sitia et al., 1990). If, in these truncated μs molecules, the cysteine residue in the tailpiece is mutated, both mechanisms of retention are now lost and the truncated μs no longer binds BiP and is secreted. In virgin B cells μs2L2 tetramers are retained intracellularly via the exposed cysteine containing tailpiece. Later in differentiation, the process of IgM polymerization masks the tailpiece retention region and pentameric IgM is secreted.

The signals for retention present on membrane immunoglobulins are less well defined, although in the case of membrane IgM recent studies have suggested that one such signal may reside in a polar serine and threonine rich stretch in the μm transmembrane domain (Williams et al., 1990). Another possible retention signal on membrane immunoglobulin heavy chains is the CH1 domain, which is the absence of being masked by a light chain may function in a manner similar to the identical region on secretory heavy chains. Indeed it is generally assumed that the CH1 domain plays as important a role in the retention of membrane heavy chains as it does in the retention of their secretory counterparts.

We wished to examine the nature of the retention signals in membrane and secretory heavy chain immunoglobulins by comparing the fate of immunoglobulins that have a similar retention motif (an exposed CH1 region) in their extracellular domains and differ only in the presence or absence of membrane anchors. We studied the biosynthesis and fate of

these proteins in 18-81A2 (Burrows et al., 1983), a pre-B Abelson cell line that has switched to the γ isotype and which does not synthesize either the conventional κ or λ or the surrogate ω light chain proteins. We show here that γm in this cell line is rapidly degraded, while γs (which lacks the CH4 tailpiece found in μs) is retained intracellularly bound to BiP, presumably through the CH1 domain. In a transfectant expressing a relative excess of κ light chain protein, γs2κ2 tetramers are efficiently secreted; although γm can associate with κ it is nonetheless rapidly degraded. The specific degradation of γm is temperature sensitive and is not inhibited by lysosomotropic agents; this protein does not acquire resistance to Endo H before degradation, suggesting that proteolysis occurs in a pre-Golgi compartment. The rapid degradation of membrane as opposed to secretory immunoglobulins suggests that signals besides the CH1 domain may determine the fate of incomplete membrane immunoglobulin oligomers; this degradatory process may itself function as a retention mechanism. The role of putative γ isotype specific associated proteins, hydrophilicity of the transmembrane domain and the mechanisms involved in membrane immunoglobulin transport at the pre-B to B cell transition are discussed.

Materials and Methods

Reagents

Endo-β-*N*-acetylglucosaminidase H (Endo H) was obtained from New England Nuclear (Boston, MA); PMSF, methionine, chloroquine, and monensin were obtained from Sigma Chemical Co. (St. Louis, MO); protein A-Sepharose was obtained from Pharmacia Inc. (Piscataway, NJ); anti-κ Sepharose was from Zymed Laboratories Inc. (South San Francisco, CA); and Brefeldin A was a gift from Sandoz Ltd. (Basel, Switzerland).

Cells

18-81A2 is a cell line that was isolated as a derivative of an AMuLV transformed pre-B cell line that underwent an isotypic switch of the heavy chain from μ to γ2b but has an unrearranged κ locus (Burrows et al., 1983). 18-81A2κ2 is a derivative of 18-81A2 that expresses a transfected κ light chain gene (Rice and Baltimore, 1983).

Metabolic Labeling and Immunoprecipitation

Cells were metabolically pulse labeled and chased as described previously (Pillai and Baltimore, 1987*b*). 0.5% Triton X-100 in reticulocyte saline buffer (RSB; 10 mM Tris pH 7.5, 3 mM $MgCl_2$, 10 mM NaCl) containing 2 mM PMSF was used as the lysis buffer. Lysates were immunoprecipitated by mixing overnight with 180 μl of a 3% slurry of protein A-Sepharose. Samples were analyzed by electrophoresis on 9% polyacrylamide-sodium dodecyl sulfate gels.

Metabolic Labeling in the Presence of Inhibitors

In experiments where the effects of different inhibitors were investigated, the inhibitors were added 60 min before the labeling period (during methionine starvation) and were present during the labeling and chase periods.

Endo H Treatment

Washed protein A-Sepharose beads were boiled in 30 μl of 100 mM sodium citrate buffer, pH 5.5, containing 0.4% SDS for 5 min, diluted twofold with water, centrifuged, and the supernatant divided into two aliquots. To one aliquot 75 ng of Endo H was added while the other aliquot served as a control. Both tubes were incubated at 37°C for 16 h.

Surface Iodination

Live cells were iodinated as described previously (Pillai and Baltimore, 1987*b*).

Results

Rapid Turnover of γm, but Not γs, in 18-81A2 Cells

Although γm and γs were synthesized in almost equal amounts in 18-81A2 cells, γm underwent rapid intracellular degradation and was barely detectable after 4 h of chase (Fig. 1). In contrast γs was retained intracellularly and exhibited a relatively long half-life. The coprecipitation of BiP with γs (and BiP being known to be an ER protein) suggested that γs was retained in the ER. The γs protein was not detectable in the cell supernatants, presumably due to its inability to form functional oligomers in the absence of light chain, and its consequent retention in association with BiP.

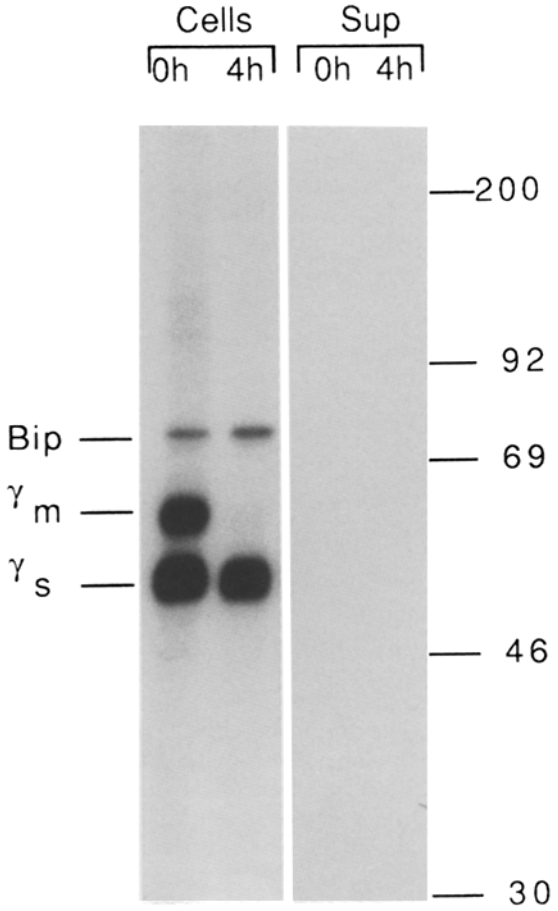

Figure 1. Fate of γm and γs in 18-81A2 cells. Cells were metabolically labeled, chased, and immunoprecipitated as described in the text. Samples were analyzed on a 9% polyacrylamide-sodium dodecyl sulfate gel. *Sup* refers to cell supernatants. *0 h* and *4 h* refer to chase times in hours after the labeling period. Position of molecular mass markers (in kilodaltons; Rainbow markers; Amersham Corp., Arlington Heights, IL) are indicated on the right.

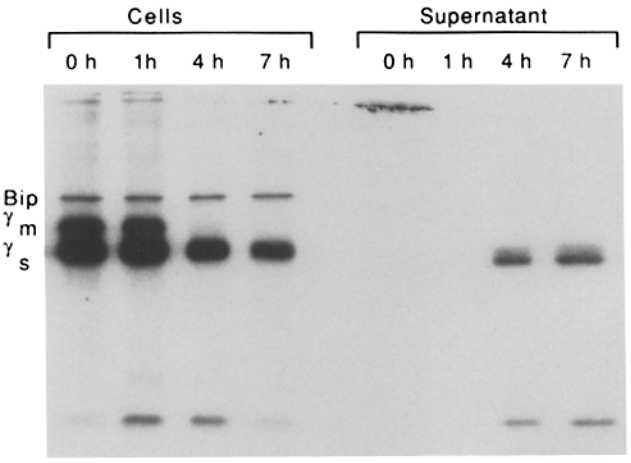

Figure 2. Fate of γm and γs in 18-81A2κ2 cells. See legend to Fig. 1 and text for details.

Intracellular Fate of γm and γs in 18-81A2κ2 Cells

The 18-81A2κ2 transfectant cell line expresses a rearranged κ-light chain gene which associates with the endogenous γ heavy chain (Rice and Baltimore, 1983). Anti-κ-Sepharose precipitated both γm and γs in this cell line (data not shown) confirming that a functional κ light chain was being made.

An examination of the fate of γm and γs in 18-81A2κ2 revealed that while γm was degraded at virtually the same rate as in the parent cell line, γs, in association with κ, formed disulfide linked tetramers that were secreted (Fig. 2). Cell surface iodination studies on 18-81A2κ2 failed to reveal any γm on the surface of these cells (data not shown).

The Effect of Inhibitors of Lysosomal Function on γm Degradation

To understand the nature of the γm degradation pathway we studied the effect of different inhibitors on the degradation of γm. NH4Cl and chloroquine are lysosomotropic agents that interfere with lysosomal activity by raising the pH of these organelles (Poole and Ohkuma, 1981). These drugs did not lead to any detectable inhibition of γm degradation (Fig. 3). Monensin is a carboxylic ionophore that is known to interfere with ER to Golgi compartment and primarily intra-Golgi transport (Tartakoff, 1983). Concentrations of monensin that significantly inhibited secretion of γs2κ2 in 18-81Aκ2 cells, did not lead to significant inhibition of degradation of γm in either 18-81A2 or 18-81A2κ2 (Fig. 3). These experiments suggested that γm degradation was non-lysosomal and probably occurred before entry into the Golgi compartment either in the ER or in a post-ER, pre-Golgi compartment.

Endo H Sensitivity of γm and γs

To examine whether γm degradation occurred after passage through the Golgi compartment, we investigated the Endo H sensitivity of γm. Proteins in the ER which have high mannose residues are sensitive to Endo H, an enzyme that cleaves the chitobiose core (Tarentino and Maley, 1974). In proteins that have reached the Golgi compartment, peripheral mannose residues are trimmed and an *N*-acetylglucosamine moiety is added in the medial Golgi stack which renders these proteins resistant to Endo H. When we examined the sensitivity of γm and γs to Endo H in 18-81A2 cells, both of the proteins were sensitive to Endo H (Fig. 4). The γs protein continued to remain Endo H sensitive with time and the

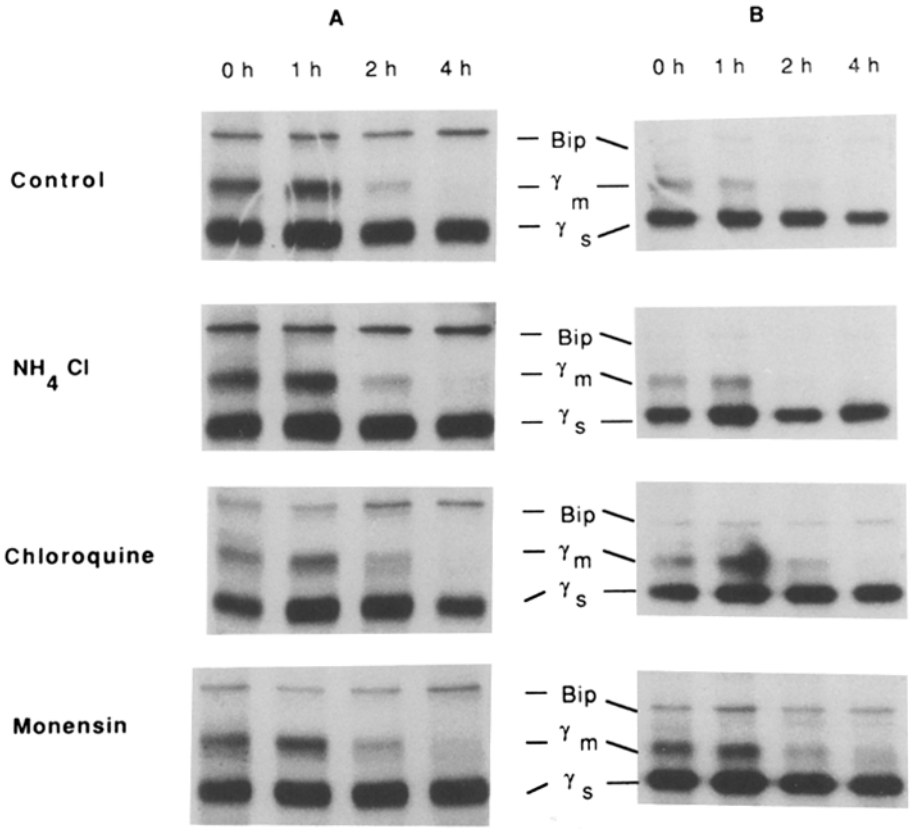

Figure 3. Effect on drugs on the degradation of γm. 18-81A2 and 18-81A2κ2 cells were starved, metabolically labeled, and chased as described in the text in medium containing either NH4Cl (50 mM), Chloroquine (100 μM) or monensin (7 μg/ml). Control cells received no drugs. Cells were processed and analyzed as described in legend to Fig. 1. (*A*) 18-81A2; (*B*) 18-81A2κ2.

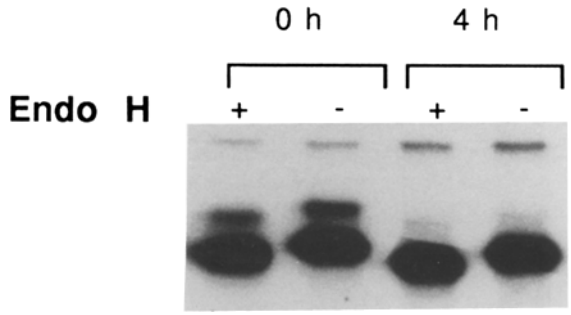

Figure 4. Endo H sensitivity of γm and γs in 18-81A2 cells. 18-81A2κ2 cells were metabolically labeled and chased for 4 h in medium containing excess unlabeled methionine. Immunoprecipitates were treated (+) with Endo H as described in the text and analyzed in parallel with mock-treated (−) samples as described in the legend to Fig. 1.

residual γm that could be detected by overexposure of the autoradiograms revealed that this protein remains Endo H sensitive (Fig. 4). This suggests that the degradation of γm occurs before its entry into the Golgi.

Effect of Temperature and Brefeldin A on γm Degradation

Intracellular processes that are dependant on membrane fusion have been shown to be temperature dependent (Hough and Rechsteiner, 1984). Temperatures below 16°C block movement of proteins out of the ER, while temperatures below 20°C inhibit movement from the *trans*-Golgi to the cell surface (Saraste et al., 1986). We examined the effect of temperature on γm degradation. 18-81A2 cells were pulse-labeled at 37°C and chased at different temperatures. Degradation of γm was almost totally inhibited even at 24°C (Fig. 5), a temperature at which exit from the ER is not inhibited. This suggests that γm degradation is extremely sensitive to temperature, an observation made previously for other proteins that are rapidly degraded in the ER (Lippincott-Schwartz et al., 1988; Amara et al., 1989; Koppelman and Cresswell, 1990).

Brefeldin A, a fatty acid derivative that inhibits protein secretion, blocks ER to Golgi transport (Takatsuki and Tamura, 1985; Misumi et al., 1986), without inhibiting retrograde transport and fusion of the Golgi with the ER (Lippincott-Scwartz et al., 1989; Doms et al., 1989; Orci et al. 1991). In our experiments, Brefeldin A partially inhibited γm degradation (Fig. 6). The acquisition of Endo H resistance by both γm and γs (data not shown) suggests that the ER compartment was drastically altered in its biochemical

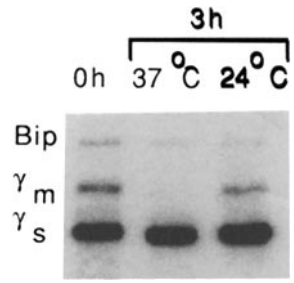

Figure 5. Effect of temperature on γm degradation. Cells were metabolically labeled for 15 min at 37°C and chased in excess cold methionine at either 37 or 24°C. Samples were analyzed as described in the legend to Fig. 1.

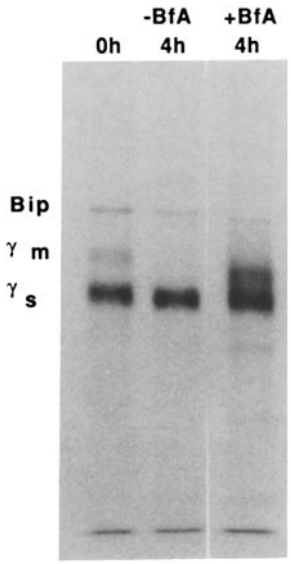

Figure 6. Effect of Brefeldin A on γm degradation. Cells were metabolically labeled for 15 min at 37°C and chased in medium containing excess unlabeled methionine with or without Brefeldin A (10 μg/ml).

properties during the process of fusion with the Golgi. These biochemical alterations could account for the inhibition of the process of γm degradation.

Discussion

We have described the rapid and specific pre-Golgi degradation of γm but not γs in a pre-B cell line. The fate of γm could not be altered by association with a light chain suggesting that the presence of an exposed CH1 domain has little effect on the fate of membrane immunoglobulin heavy chains. In contrast, γs, which was intracellularly retained in association with BiP, had a dramatically different fate when allowed to associate with light chains and was secreted as γs2κ2 tetramers. This also indicated that in contrast to earlier suggestions (Sitia et al., 1987) early B lineage cells are competent for immunoglobulin secretion although they might lack the capacity to polymerize IgM.

Rapid degradation in a pre-Golgi compartment has been described in some detail in the case of the T cell receptor (TCR)[1]-α chain expressed alone in transfected non lymphoid cell lines, or in T cell hybridomas which lack associated TCR proteins (Lippincott-Schwartz et al., 1988). Similar findings have been described for the TCR-β and TCR-δ chains (Chen et al., 1988) as well as for a few other proteins (Amara et al., 1989; Koppelman and Cresswell, 1990; Klausner and Sitia, 1990). In all cases degradation occurred rapidly after a brief lag phase, and was sensitive to temperature.

Recent studies on the TCR-α chain have localized the targeting signal to a 9-amino acid stretch in the transmembrane domain (Bonifacino et al., 1990*a*). Two charged amino acids within this 9-amino acid stretch appear to be the major determinants for this process as mutating these residues to hydrophobic amino acids leads to protection from degradation (Bonifacino et al., 1990*b*). In the case of the asialoglycoprotein H2a subunit however, the signal for degradation was found to reside in a 5-amino acid stretch of the extracellular

1. *Abbreviation used in this paper*: TCR, T cell receptor.

domain just adjacent to the transmembrane domain (Lederkremer and Lodish, 1991). The γm protein differs from γs in having a short additional extracellular stretch, a transmembrane domain and a cytoplasmic tail. The signals for the degradation of γm should therefore reside within this region which is encoded by the two membrane exons.

The signals for rapid degradation of the TCR-α chain colocalize with transmembrane segments that play a role in the process of assembly with the other subunits of the T cell receptor complex (Bonifacino et al., 1990). Since assembly with the other subunits protects TCR-α from degradation (Bonifacino et al., 1989, 1990; Wileman et al., 1990) it is possible that the transmembrane domain of membrane immunoglobulins (which contain polar amino acids but no charged residues) may serve as a signal for degradation as well as for assembly with associated proteins. It is interesting to note in this context that mutating a hydrophilic pentapeptide stretch in the μm transmembrane domain to a more hydrophobic segment resulted in the ability of μm to reach the cell surface (Williams et al., 1990) along with light chains in nonlymphoid cells (which lack B29 and mb-1). Transport of μm-light chain tetramers occurs only in B cells in association with the B29 and mb-1 proteins. The hydrophilic TTAST stretch in μm may be viewed as a retention signal which might be masked by the products of the mb-1 and B29 genes. The same sequence may act as a signal for both assembly with the associated proteins and as a signal for degradation in the absence of assembly.

Considerable progress has been made in identifying proteins associated with membrane immunoglobulins that are essential for their transport to the cell surface. These include the IgM(α) protein which is the product of the mb-1 gene (Hombach et al., 1988*a*, *b*; Sakaguchi et al., 1988) and the Ig(β) protein which is the product of the B29 gene (Hombach et al., 1990*b*; Hermanson et al., 1988). A number of additional proteins have been demonstrated to be associated with μm in pre-B (Pillai and Baltimore, 1988; Takemori et al., 1990) and in B cells (Hombach et al., 1990; Wienands et al., 1990). One of these, the product of the mb-1 gene, is essential for the surface transport of μm in mature B cells. Associated proteins specific for the δm isotype have also been demonstrated (Wienands et al., 1990; Chen et al., 1990) and the existence of other isotype specific proteins (which may be relevant to the expression of these immunoglobulins on the cell surface) has been postulated (Wienands et al., 1990). Although the mb-1 and B29 genes are expressed from the earliest stages of B cell ontogeny, genes for other isotype specific proteins remain to be cloned and it is unclear if the expression of the proteins they encode is developmentally regulated.

A key event at the pre-B to B cell transition is the acquisition of the ability to transport μm to the cell surface. Transport of μm to the cell surface does not depend upon association with a conventional κ or λ light chain; in a subset of pre-B cell lines μm can be transported to the cell surface in the absence of κ or λ light chains (Paige et al., 1981; Findley et al., 1981; Gordon et al., 1983; Hendershot and Levitt, 1984; Hardy et al., 1986). In these cell lines and in transitional B cells in the bone marrow (which synthesize both surrogate and conventional light chains), the heavy surrogate light chain complex is transported to the cell surface (Pillai and Baltimore, 1987*a*, 1988; Kerr et al., 1989; Cherayil and Pillai, 1991). The molecular basis for the acquisition of the ability to transport μm to the cell surface at the pre-B to B cell transition is unlikely to be the activation of the mb-1 or the mb-1 like B 29 gene, since both these genes are expressed in all pre-B and B cells including cells in the process of rearranging their H-chain loci (Sakaguchi et al., 1987; Hermanson et al., 1988). The transport of μm to the cell surface at the pre-B to B cell transition has also been correlated with the posttransitional acquisition of hydrophobicity by the μm polypeptide (Pillai and Baltimore, 1987*b*). The inability of 18-81A2κ2 cells to transport γm to the cell surface even as they secrete γs may therefore either reflect their lack of some (possibly γ isotype specific) associated protein or else the lack of some other posttranslational modification that would protect this membrane immunoglobulin heavy chain from degradation.

We thank Douglas Rice and David Baltimore for the 18-81A2κ2 cell line and Bobby Cherayil for many helpful discussions.

This work was supported by a grant from the Arthritis Foundation and grant AI-27385 from the National Institutes of Health.

Received for publication 13 December 1990 and in revised form 23 April 1991.

References

Amara, J. F., G. Lederkremer, and H. F. Lodish. 1989. Intracellular degradation of unassembled asialoglycoprotein receptor subunits: a pre-Golgi, nonlysosomal endoproteolytic cleavage. *J. Cell Biol.* 109:3315–3324.

Bole, D. G., L. Hendershot, and J. F. Kearney. 1986. Posttranslational association of immunoglobulin heavy chain binding protein with nascent heavy chains in nonsecreting and secreting hybridomas. *J. Cell Biol.* 102:1558–1566.

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

Bonifacino, J. S., P. Cosson, and R. D. Klausner. 1990. Colocalized transmembrane determinants for ER degradation and subunit assembly explain the intracellular fate of TCR chains. *Cell.* 63:503–513.

Burrows, P. D., G. B. Beck-Engeser, and M. R. Wabl. 1983. Immunoglobulin heavy chain class switching in a pre-B cell line is accompanied by DNA rearrangement. *Nature (Lond.).* 306:243–246.

Chen, C., J. S. Bonafacino, L. C. Yuan, and R. D. Klausner. 1988. Selective degradation of T cell antigen receptor chains in a pre-Golgi compartment. *J. Cell Biol.* 107:2149–2161.

Chen, J., A. M. Stall, L. A. Herzenberg, and L. A. Herzenberg. 1990. Differences in glycoprotein complexes associated with IgM and IgD in normal murine B cells potentially enable transduction of different signals. *EMBO (Eur. Mol. Biol. Organ.) J.* 9:2117–2121.

Cherayil, B. J., and S. Pillai. 1991. μm is associated with the ω/λ5 surrogate immunoglobulin light chain on the surface of bone-marrow pre-B lymphocytes. *J. Exp. Med.* 173:111–116.

Doms, R. W., G. Russ, and J. W. Yewdell. 1989. Brefeldin A redistributes resident and itinerant Golgi proteins to the endoplasmic reticulum. *J. Cell Biol.* 109:61–72.

Findley, H. W., M. D. Cooper, J. H. Kim, C. Alvardo, and A. H. Ragab. 1982. Two new acute lymphoblastic leukemic cell lines with early B-cell phenotypes. *Blood.* 60:1305–1309.

Gething, M.-J., K. McCammon, and J. Sambrook. 1986. Expression of wild-type and mutant forms of influenza hemagglutinin: the role of folding in intracellular transport. *Cell.* 46:939–950.

Gordon, J., T. J. Hamblin, J. L. Smith, F. K. Stevenson, and G. T. Stevenson. 1981. A human B-cell lymphoma synthesizing and expressing surface μ-chain in the absence of detectable light chain. *Blood.* 58:552–556.

Haas, I. G., and M. R. Wabl. 1983. Immunoglobulin heavy chain binding protein. *Nature (Lond.).* 306:387–389.

Hendershot, L. 1990. Immunoglobulin heavy chain binding protein complexes are dissociated in vivo by light chain addition. *J. Cell Biol.* 111:829–837.

Hendershot, L., and D. Levitt. 1984. Analysis of surface μ-chain expression in human lymphoblastoid cell lines that do not produce light chains. *J. Immunol.* 132:502–509.

Hendershot, L., D. Bole, G. Kohler, and J. F. Kearney. 1987. Assembly and secretion of heavy chains that do not associate post-translationally with immunoglobulin heavy chain binding protein. *J. Cell Biol.* 104:761–767.

Hermanson, G. G., D. Eisenberg, P. W. Kincade, and R. Wall. 1988. B29:

a member of the immunoglobulin gene superfamily exclusively expressed on B-cell lineage cells. *Proc. Natl. Acad. Sci. USA.* 85:6890–6894.

Hombach, J., T. Tsubata, L. Leclerq, H. Stappert, and M. Reth. 1990*a*. Molecular components of the B-cell antigen receptor complex of the IgM class. *Nature (Lond.).* 343:760–762.

Hombach, J., F. Lottspeich, and M. Reth. 1990*b*. Identification of the genes encoding the IgM-α and Ig-β components of the IgM antigen receptor complex by amino-terminal sequencing. *Eur. J. Immunol.* 20:2795–2799.

Hurtley, S. M., and A. Helenius. 1989. Protein oligomerization in the endoplasmic reticulum. *Annu. Rev. Cell Biol.* 5:277–307.

Kashiwamura, S., T. Koyama, T. Matsuo, M. Steinmetz, M. Kimoto, and N. Sakaguchi. 1990. Structure of the murine mb-1 gene encoding a putative sIgM associated molecule. *J. Immunol.* 145:337–343.

Kerr, W. G., M. D. Cooper, L. Feng, P. D. Burrows, and L. M. Hendershot. 1989. Mu heavy chains can associate with a pseudo-light chain complex (σL) in human pre-B cell lines. *Int. Immunol.* 4:355–361.

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

Koppelman, B., and P. Cresswell. 1990. Rapid, nonlysosomal degradation of assembled HLA class II glycoproteins incorporating a mutant DR-α chain. *J. Immunol.* 145:2730–2736.

Kreis, T. E., and H. F. Lodish. 1986. Oligomerization is essential for transport of vesicular stomatitis viral glycoprotein to the cell surface. *Cell.* 46:929–937.

Lederkremer, G. Z., and H. F. Lodish. 1991. An alternately spliced miniexon alters the subcellular fate of the human asialoglycoprotein H2 subunit. *J. Biol. Chem.* 266:1237–1244.

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

Lippincott-Schwartz, J., L. C. Yuan, J. S. Bonafacino, and R. D. Klausner. 1989. Rapid redistribution of Golgi proteins into the ER in cells treated with Brefeldin A: evidence for membrane cycling from Golgi to ER. *Cell.* 56:801–813.

Lippincott-Schwartz, J., J. G. Donaldson, A. Schweizer, E. G. Berger, H.-P. Hauri, L. C. Yuan, and R. D. Klausner. 1990. Microtubule dependant retrograde transport of proteins into the ER in the presence of Brefeldin A suggests an ER recycling pathway. *Cell.* 60:821–836.

Misumi, Y., Y. Misumi, K. Miki, A. Takatsuki, G. Tamura, and Y. Ikehara. 1986. Novel blockade by Brefeldin A of intracellular transport of secretory proteins in cultured rat hepatocytes. *J. Biol. Chem.* 261:11398–11403.

Morrison, S. L., and M. D. Schraff. 1975. Heavy chain producing variants of a mouse myeloma cell line. *J. Immunol.* 114:655–659.

Munro, S., and H. R. B. Pelham. 1986. A C-terminal signal prevents secretion of luminal ER proteins. *Cell.* 48:899–907.

Orci, L., M. Tagaya, M. Amherdt, A. Perrelet, J. G. Donaldson, J. Lippincott-Schwartz, R. D. Klausner, and J. E. Rothman. 1991. Brefeldin A, a drug that blocks secretion, prevents the assembly of non-clathrin-coated buds on Golgi cisternae. *Cell.* 64:1183–1195.

Paige, C. J., P. W. Kincade, P. Ralph. 1981. Independent control of immunoglobulin heavy and light chain expression in a murine pre-B-cell line. *Nature (Lond.).* 292:631–633.

Pelham, H. R. B. 1989. Control of protein exit from the endoplasmic reticulum. *Annu. Rev. Cell Biol.* 5:1–23.

Pillai, S., and D. Baltimore. 1987*a*. Formation of disulfide linked $\mu 2\omega 2$ tetramers in pre-B cells by the 18K ω-immunoglobulin light chain. *Nature (Lond.).* 329:172–174.

Pillai, S., and D. Baltimore. 1987*b*. Myristoylation and the posttranslational acquisition of hydrophobicity of the membrane immunoglobulin heavy chain polypeptides in B-lymphocytes. *Proc. Natl. Acad. Sci. USA.* 84:7654–7658.

Pillai, S., and D. Baltimore. 1988. The omega and iota surrogate light chain. *Curr. Top. Microb. Immunol.* 137:136–139.

Poole, B., and S. Ohkuma. 1981. Effect of weak bases on the intralysosomal pit in mouse peritoneal macrophages. *J. Cell Biol.* 90:665–669.

Rice, D., and D. Baltimore. 1982. Regulated expression of an immunoglobulin κ gene introduced into a mouse lymphoid cell line. *Proc. Natl. Acad. Sci. USA.* 79:7862–7865.

Sakaguchi, N., S. Kashiwamura, M. Kimoto, P. Thalmann, and F. Melchers. 1988. B-lymphocyte lineage restricted expression of mb-1, a gene with CD-2 like structural properties. *EMBO (Eur. Mol. Biol. Organ.) J.* 7:3457–3464.

Saraste, J., G. E. Palade, and M. G. Farquhar. 1986. Temperature sensitive steps in the transport of secretory proteins through the Golgi complex in exocrine pancreatic cells. *Proc. Natl. Acad. Sci. USA.* 83:6425–6430.

Sitia, R., M. Neuberger, and C. Milstein. 1987. Regulation of membrane IgM expression in secretory B cells: translational and posttranslational events. *EMBO (Eur. Mol. Biol. Organ.) J.* 6:3969–3977.

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

Takatsuki, A., and G. Tamura. 1985. Brefeldin A, a specific inhibitor of intracellular translocation of vesicular stomatitis virus G protein: intracellular accumulation of high mannose type G protein and inhibition of its cell surface expression. *Agric. Biol. Chem.* 48:899–902.

Takemori, T., J. Mizuguchi, I. Miyazoe, M. Nakanishi, K. Shigemoto, H. Kimoto, T. Shirasawa, N. Maruyama, and Taniguchi. 1990. Two types of μ chain complexes are expressed during differentiation from pre-B to mature B cells. *EMBO (Eur. Mol. Biol. Organ.) J.* 9:2493–2500.

Tarentino, A. L., and F. Maley. 1974. Purification and properties of an endo-β-acetyl-glucosaminidase from Streptomyces griseus. *J. Biol. Chem.* 249:811–816.

Tartakoff, A. M., 1983. Perturbation of vesicular traffic with the carboxyBic ionophore, monensin. *Cell.* 32:1026–1028.

Wielands, J., J. Hombach, A. Radbruch, C. Riasterer, and M. R. Reth. 1990. Molecular components of the B-cell antigen receptor complex of class IgD differs partly from those of IgM. *EMBO (Eur. Mol. Biol. Organ.) J.* 9:449–455.

Wileman, T., G. R. Carson, M. Concino, A. Ahmed, and C. Terhorst. 1990. The γ and ϵ subunits of the CD3 complex inhibit pre-Golgi degradation of newly synthesized T cell antigen receptors. *J. Cell Biol.* 110:973–986.

Williams, G. T., A. R. Venkitaraman, D. J. Gilmore, and M. S. Neuberger. 1990. The sequence of the μ transmembrane segment determines the tissue specificity of the transport of immunoglobulin M to the cell surface. *J. Exp. Med.* 171:947–952.