Immunoglobulin Light Chains Dictate Vesicular Transport-dependent and -independent Routes for IgM Degradation by the Ubiquitin-Proteasome Pathway*

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Degradation of IgM μ heavy chains in light chain-negative pre-B cells is independent of vesicular transport, as is evident by its insensitivity to brefeldin A or cell permeabilization. Conversely, by the same criteria, degradation of the secretory μs heavy chain in light chain-expressing B cells depends on vesicular transport. To investigate whether the presence of conventional light chains or the developmental stage of the B-lymphocytes dictates the degenerative route taken by μ, we express in 70Z/3 pre-B cells either λ ectopically or κ by lipopolysaccharides-stimulated differentiation into B cells and show their assembly with μ heavy chains. The resulting sensitivity of μ degradation to brefeldin A and cell permeabilization demonstrates that conventional light chains, a hallmark of B cell differentiation, are necessary and sufficient to divert μ from a vesicular transport-independent to a vesicular transport-dependent degradative route. Although both routes converge at the ubiquitin-proteasome degradation pathway, only in light chain-expressing cells is vesicular transport a prerequisite for μ ubiquitination.

Differentiation of B-lymphocytes is accompanied by a regulated switch in the expression pattern and stability of surface and secretory Igs. The secretory form of IgM (sIgM), a stable molecule that is efficiently secreted from plasma cells, is rapidly degraded at the earlier differentiation stages of pre-B and B cells (1–3). In the 38C B cell line, this degradation is selective to the sIgM that contains the secretory heavy chain μs, whereas the very similar membrane form of IgM that contains the membrane heavy chain μm is stable and displayed at the cell surface (1, 4, 5). This selective sIgM degradation is nonsosomally and occurs prior to the trans-Golgi (1), yet it requires vesicular transport from the endoplasmic reticulum (ER) (2–4). This implicates quality control mechanism(s) not only asso-
EXPERIMENTAL PROCEDURES

Cell Lines—The murine 70Z/3 pre-B cell line (American Type Culture Collection; catalog number TIB-158), and the 38C B-lymphocyte cell line were previously described (1). 70Z/3 cells were cultured to produce light chain by an 18-h incubation with 5 μg/ml LPS (Sigma) (15). The degree of differentiation into B cells was estimated by cytofluorometric analyses, staining the cells with R-6-60.2 anti-IgM antibody (Pharmingen).

Ectopic Expression in 70Z/3 Cells of a Light Chain Using a Vaccinia Virus T7 RNA Polymerase Hybrid System—The vaccinia virus/T7 RNA polymerase overexpression hybrid system (16) was used. AIFcDNA was generated by reverse transcribing RNA from COS-7 cells transiently transfected with pJDEAI genomic AIF vector (kindly provided by Y. Argon), followed by PCR using PWO DNA polymerase (Roche Applied Science) and these forward (5’-CCATGCAATGCGTCTTCTTATC-TTATAC-3’) and reverse (5’-TCTCCCCGGGCGCTAAGCAGTCCG-ACGG-3’) primers. The AIFcDNA was cloned between the NcoI and SmaI sites of pTM1 (17), and its authenticity was verified by DNA sequencing.

Preparation of AI-encoding vaccinia virus was according to Current Protocols in Molecular Biology (18). Confluent HuTK 143B cells, maintained in Dulbecco’s modified Eagle’s medium supplemented with 5% fetal calf serum, 50 units/ml penicillin, and 50 μg/ml streptomycin, were infected with WR wild-type vaccinia virus and 4 h later were transfected with the pTM1-AIF. Thirty minutes later, the cells were covered with fresh medium that was also supplemented with 5 μg/ml bromodeoxyuridine and 100 μg/ml Neutral Red (Sigma) and in which low melting agarose (Amresco) was dissolved to 1%. Reconstituent bromodeoxyuridine was added to the plates that were incubated and tested for the presence of AIFcDNA with the primers described above. Positive clones were enriched and purified by three additional cycles of infection, plaque selection, and PCR testing. Large scale preparation of AI-encoding vaccinia virus were made in Hela cells 3 days post-infection, and viral titer were estimated by serial dilution and infection of CV-1 cells, followed by crystal violet staining. Viral stocks were stored at −80°C.

A 20 multiplicity of infection of AI or WR (“mock”) viruses along with a 10 multiplicity of infection of T7 RNA polymerase viruses were mixed with an equal volume of trypsin (0.25 mg/ml; Worthington). The mixtures were incubated for 30 min at 37°C, sonicated in a bath sonicator on ice (three bursts of 10 s each), and then immediately added to 70Z/3 cells (106 cells in 0.5 ml of RPMI supplemented with 2.5% fetal calf serum). After 3 h of continuous agitation at 37°C, the cells were diluted 20-fold to 106 cells/ml with the same medium, and the infected cells were assayed 10–13 h later.

 Biosynthetic Labeling, Cell Permeabilization, and in Vitro Incubation—The cells were pulse-labeled with [35S]methionine and either chased in vitro (1) or permeabilized with 55 μg/ml digitonin (Calbiochem), resuspended in growth medium and chased in vitro (3). Where indicated, BFA, N-acetyl-leucyl-leucyl-norleucine (ALLN), carboxybenzyl-leucyl-leucyl-leucinal (MG-122), carboxybenzyl-leucyl-leucyl-leucinal vinyl sulfone (Z-L3VS), or dithiothreitol were added. The ALLN (100 μM; Calbiochem), MG-122 (5 μM; Calbiochem), BFA (5 μg/ml; Epicentre), or dithiothreitol (5 mM) was added to the chase medium, whereas Z-L3VS (5 μM) was present during the starvation, pulse, and chase.

Immunoprecipitation, Immunoblotting, Lectin Blotting, and Treatment with Endoglycosidase H—IgM was immunoprecipitated from lysates obtained from an identical number of intact or permeabilized cells, normalized for equal cell protein and equal 35S incorporation into total cell protein. To ensure immunoprecipitation of the entire IgM, an excess of goat anti-mouse IgM antibodies was used, followed by protein A-Sepharose (Repligen). Immunoprecipitated µ or total cell proteins were resolved by reducing or nonreducing SDS-PAGE and electrophoretically blotted onto nitrocellulose, and the blots were probed with the indicated antibodies. When radioabeled proteins were resolved, the gels were either directly autoradiographed or first electroblotted, and then the blots were autoradiographed and subsequently probed. The latter protocol, which is routinely used and is represented here in Fig. 5B, confirms that at each time point throughout the chase, identical steady-state amounts of µ protein are immunoprecipitated, which include the radiolaabeled µ. This indicates that the decrease with time in radiolabeled µ reflects a net loss of intracellular µ. Incubation of immunoprecipitated IgM with endoglycosidase H (New England Biolabs) was as previously described (1). Assembly of µ with either or λ or µ was verified by the positive staining of anti-IgM immunoprecipitates resolved by nonreducing SDS-PAGE with both anti-µ and either anti-κ or anti-λ antibodies. Assembly efficiency of µ with λ, κ, or both (mixed popula-

RESULTS

κ Light Chain Assembles with µ Heavy Chain, Displaces BiP, and Enables Maturation and Surface Expression of µm—The intracellular fate of IgM during B cell differentiation was followed in the well characterized 70Z/3 cell line (15). In unstimulated 70Z/3 cells, the majority of µ heavy chains were found either free or engaged in µκ homodimers (Fig. 1A, lane 3). Although low levels of hemimers composed of µ and λ5 surrogate light chain (SLC) were observed, higher assembly forms of µλ52 monomers were not found (Fig. 1A, lane 3). Expression of κ light chain was not detected in unstimulated 70Z/3 cells (Fig. 1A, lane 6). Upon LPS stimulation, induction of κ expression resulted in efficient formation of µκ hemimers, which further assembled into µκκ2 monomers, as detected both by anti-µ (Fig. 1A, lane 2) and anti-κ (Fig. 1A, lane 5) antibodies, and the levels of µκ hemimers were greatly diminished (Fig. 1A, lane 2). Interestingly, despite the relatively large quantities of µκκ2 monomers, minute amounts of SLC-containing µλ5 hemimers were still detected (Fig. 1A, lane 2).

The expression of κ and its assembly with µ altered the intracellular fate of the latter, resulting in considerable maturation and cell surface expression of the membrane µκm. This was indicated by the retarded mobility (Fig. 1B, lane 2), the increase from 18 to 42% of µ that acquired resistance to endoglycosidase H (Fig. 1C, compare lanes 1 and 3), the galactosylation of µ in the trans-Golgi detected by lectin blotting only in LPS-stimulated 70Z/3 cells (Fig. 1D, compare lanes 1 and 2), and the increase in surface membrane form of IgM from 14% in unstimulated 70Z/3 cells to 76% in LPS-stimulated 70Z/3 cells, as detected by cyt fluorometric analysis (Fig. 1E). This indicated that only in the presence of κ, did a substantial fraction of µm traverse the entire secretory pathway to be displayed at the cell surface, where it served as a B cell receptor (20–23).

The large quantities of BiP that co-precipitated with µ (Fig. 1B, lane 3) suggested that, also in 70Z/3 pre-B cells, the intracellular retention of unassembled µ was mediated to a large extent by the interaction of µ with BiP. Indeed, upon κ expression and its assembly with µ, the levels of BiP that co-precipitated with µ markedly decreased (Fig. 1B, lane 4), even though the cellular levels of BiP were hardly affected (Fig. 1B, lanes 5 and 6). Inasmuch as κ expression was also correlated with the intracellular transport of µm to the cell surface, our results were consistent with the notion that upon differentiation, the expressed κ assembled with µ and displaced BiP.

κ Expression, the Hallmark of Pre-B to B Cell Differentiation, Diverts µ from a Vesicular Transport-independent to a Vesicular Transport-dependent Degradation—Because differentiation of 70Z/3 pre-B cells to κ-expressing B cells was manifested by modified assembly patterns, maturation, and surface expression of µm and displacement of BiP (Fig. 1), we anticipated that this developmental passage would be accompanied by a conversion in the degradation mode of the µ heavy chains. When µ stability was followed in pulse-chase experiments, t1/2 of ~55 min was measured in unstimulated 70Z/3 cells (Fig. 2, lanes

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1–3 and open circles), reflecting rapid degradation of both $\mu$s and $\mu$m. The addition of BFA during chase hardly affected $\mu$ degradation ($t_{1/2}$ of $\sim$50 min; Fig. 2, lanes 4–6 and open squares). In the LPS-stimulated cells, $\mu$ chains, which were predominantly assembled with $\kappa$ (Fig. 1A), still disappeared as rapidly, with a measured $t_{1/2}$ of $\sim$55 min (Fig. 2, lanes 10–12 and closed circles). Because assembled $\mu$m is a stable molecule (1), this half-life probably reflected degradation of assembled $\mu$s as well as of $\mu$s and $\mu$m that remained unassembled. That unassembled $\mu$s and $\mu$m represented a minor proportion of $\mu$ was indicated by the nearly arrested degradation of $\mu$ in the presence of BFA in these LPS-stimulated cells. This extension of $t_{1/2}$ to $>$9 h (Fig. 2, lanes 13–15 and closed squares) reflected that the majority of $\mu$s was indeed assembled with $\kappa$ and was therefore diverted to the vesicular transport-dependent degradation.

The change in the mode of $\mu$ degradation that accompanied pre-B to B cell differentiation was corroborated by experiments in permeabilized cells in which vesicular transport was severely hampered (3). When pulse-labeled 70Z/3 cells were permeabilized with digitonin and incubated in vitro, the stability of $\mu$ heavy chains correlated with the stage of differentiation and the expression of $\kappa$ light chain. In unstimulated $\kappa$-negative cells, $\mu$ chains were rapidly degraded during the in vitro incubations ($t_{1/2} = 54$ min; Fig. 2, lanes 7–9 and open triangles), with kinetics very similar to those measured in intact cells. Remarkably, permeabilization of LPS-stimulated $\kappa$-expressing cells resulted in stabilization of $\mu$ during the in vitro incubation, with $t_{1/2}$ significantly extended to 3.7 h (Fig. 2, lanes 16–18 and closed triangles). Taken together, these results demonstrate that upon differentiation into $\kappa$-expressing B cells, $\kappa$-assembled $\mu$ heavy chains are diverted from a BFA-insensitive transport-independent degradation to a BFA-sensitive vesicular transport-dependent process.

Ecotopically Expressed $\lambda$ Light Chain Assemblies with $\mu$ Heavy Chains and Diverts Them to a Vesicular Transport-dependent Degradation—The striking effect of the pre-B to B differentiation on the intracellular fate of $\mu$ heavy chains (Fig. 2) was not necessarily the consequence of $\kappa$ expression and its evident assembly with $\mu$ but could be simply the result of the differentiation stage, regardless of light chain expression. To test whether the mere contribution of the pre-B to B cell differentiation to the intracellular fate of $\mu$ was to provide the light chain, the latter was ecotopically expressed in pre-B cells by
infection with a light chain-encoding vaccinia virus. It allowed us to achieve light chain expression while circumventing differentiation into B cells. To verify that indeed ectopic light chain expression does not trigger differentiation into \( \kappa \)-expressing B cells, we chose to express \( \lambda \) rather than \( \kappa \). Moreover, it allowed us to generalize our observations via \( \lambda \) to \( \kappa \) the effect of conventional Ig light chains on the intracellular fate of their partner \( \mu \) heavy chains. Pulse-chase experiments following mock infection revealed a prolonged \( t_{1/2} \) of \( \mu \) chains from \(-1 \) h (Fig. 2) to \(2.5 \) h (Fig. 3A, lanes 1–4 and open circles), with BFA exerting only a slight effect (\( t_{1/2} = 3.5 \) h; Fig. 3A, lanes 5–8 and open squares). In 70Z/3 cells infected with \( \lambda \)-encoding virus, \( \mu \) chains disappeared with a somewhat extended \( t_{1/2} \) of \(3.7 \) h (Fig. 3A, lanes 9–12 and closed circles). However, when BFA was added to these \( \lambda \)-expressing 70Z/3 cells, the \( \mu \) chains were markedly stabilized with half-life extended to \( >9.5 \) h (Fig. 3A, lanes 13–16 and closed squares).

This BFA-dependent stabilization of \( \mu \) correlated with its assembly with \( \lambda \) (Fig. 3B). In mock infected 70Z/3 cells, expression of the \( \lambda \) light chain was not detected (lane 9), and the majority of \( \mu \) heavy chains were found either free, assembled into \( \mu_2 \) homodimers, or assembled into \( \lambda 5 \) SLC-containing hemimers (lane 3; see also Fig. 1A, lane 3). When cells were infected with the \( \lambda \)-encoding virus, assembly into predominant \( \mu_2 \lambda_2 \) monomers was detected, because these assembly species stained positive with both anti-\( \mu \) (lane 4) and anti-\( \lambda \) (lane 10) antibodies. Importantly, \( \lambda \) ectopic expression did not trigger differentiation into \( \kappa \)-expressing cells, based on the negative reactivity with an anti-\( \kappa \) antibody (lane 16). The assembly of \( \mu \) into \( \mu_2 \lambda_2 \) monomers was significant in \( \lambda \)-infected cells (\( \sim 50\% \) of all \( \mu \)-containing species within lane 4). On the other hand, when calculated relative to all the \( \lambda \)-containing species within the same lane (lane 10), the assembly into \( \mu_2 \lambda_2 \) reached only 15%. This was not due to inefficient assembly into \( \mu_2 \lambda_2 \) because \( \lambda \) was in excess (lane 10) and also the assembly of \( \mu \) into \( \mu_2 \kappa_2 \) monomers in LPS-treated cells did not exceed 50% when calculated relatively to all \( \mu \)-containing species within the same lane (lane 5). Note that the apparent intense band of \( \mu_2 \kappa_2 \) (lane 5) reflected the \( -2 \) fold increase in \( \mu \) expression upon LPS stimulation. Interestingly, when 70Z/3 cells were first stimulated by LPS and then infected with \( \lambda \)-encoding virus, we could detect inherent differences between the capacity of pre-B and B cells to promote assembly of \( \mu \) with \( \lambda \) into \( \mu_2 \lambda_2 \) monomers (compare lanes 10 and 12). This \( -2 \) fold improvement took place despite the vast amount of \( \kappa \) (lane 18) and the apparent preference of \( \mu \) to assemble with \( \kappa \) in B cells. This preference was manifested by the poor assembly of \( \mu \) and \( \lambda \) when \( \lambda \) was introduced into the 38C B cells, which constitutively express abundant \( \kappa \) and \( \mu \) (lane 8). Nonetheless, whenever \( \kappa \) or \( \lambda \) conventional light chains are expressed, a stabilization of \( \mu \) by

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**Fig. 3. Assembly with \( \lambda \) light chain introduced by infection diverts \( \mu \) heavy chain to BFA-sensitive vesicular transport-dependent degradation.** A, 70Z/3 cells were infected with either \( \lambda \)-encoding virus (+ and closed symbols) or mock infected with an empty vector (− and open symbols). The cells were pulse-labeled for 10 min with \( { }^{35} \text{S} \) methionine and chased for the indicated time in the absence (− and circles) or presence (+ and squares) of BFA. Immunoprecipitated IgM was resolved by reducing 10% SDS-PAGE, and \( { }^{35} \text{S} \)-labeled \( \mu \) was quantified by densitometry of autoradiograms. The plots represent the degradation of \( \mu \) heavy chains, and the remaining \( \mu \) is calculated as a percentage of its level at the end of the pulse (100%). B, unstimulated (−) or LPS-stimulated (+) 70Z/3 cells, and for comparison 38C B cells, were infected with \( \lambda \)-encoding virus (+) or mock infected (−). The proteins were resolved by nonreducing 4–12% SDS-PAGE and electroblotted, and \( \mu \) heavy chain and \( \mu \)-containing assembly intermediates were detected by an HRP-conjugated anti-\( \mu \) antibody (lanes 1–6). The \( \lambda \) light chain and \( \lambda \)-containing assembly intermediates were detected by reprobing the same blot with an HRP-conjugated anti-\( \lambda \) antibody (lanes 7–12). The \( \kappa \) light chain and \( \kappa \)-containing assembly intermediates were detected by reprobing the same blot with an HRP-conjugated anti-\( \kappa \) antibody (lanes 13–18). \( \mu \), unassembled \( \mu \) heavy chain; \( \lambda \), \( \lambda_2 \), \( \lambda \) light chain; \( \kappa \), \( \kappa \) light chain; hemimers, \( \mu \) assembled with SLC into \( \mu \lambda_5 \), with \( \lambda \) into \( \mu \lambda \), or with \( \kappa \) into \( \mu \kappa \);\( \mu_2 \) homodimers; monomers, \( \mu \) assembled with \( \lambda \) into \( \mu_2 \lambda_2 \) or with \( \kappa \) into \( \mu_2 \kappa_2 \).
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Ubiquitination of μ Heavy Chains Requires Vesicular Transport in κ-expressing B Cells but Not in Pre-B Cells—Evidently, the two degradation routes of μ heavy chains, which at their early stages were segregated into vesicular transport-dependent and -independent pathways, merged at the ultimate proteasomal degradation. To determine where along this proteasomal pathway these two routes converged, we chose to monitor the ubiquitination of μ with respect to events that required vesicular transport. Clearly, μ underwent ubiquitination along both degradation routes, as in 38C B cells (Fig. 5A), as well as in untreated or LPS-stimulated 70Z/3 cells (Fig. 5B), we detected μ heavy chains that were immunoprecipitated with anti-IgM antibody and stained positive when probed with an anti-ubiquitin antibody. As expected, ubiquitinated μ was detected in both cell types only when accumulated because of inhibition of proteasomal degradation with ALLN (Fig. 5, A, lane 2, and B, lanes 3 and 7). Conversely, when BFA was added to these proteasome-inhibited cells, this vesicular transport blocker exerted very different effects on the ubiquitination of μ in the different cell types. In the κ-expressing 38C B cells, BFA strongly attenuated the ubiquitination of μ (Fig. 5A, lanes 2 and 4), as it did to the degradation of μ (2–4), without affecting ubiquitin conjugation to cellular proteins in general (Fig. 5A, lanes 5–8). In contrast, BFA hardly affected the ubiquitination of unassembled μ in the κ-negative 70Z/3 pre-B cells (Fig. 5B, top panel, lanes 3 and 4), but again, BFA attenuated the ubiquitination of μ upon LPS-stimulated κ-expression in these cells (Fig. 5B, top panel, lanes 7 and 8). As shown in Fig. 5B (compare lanes 3 and 7 with lanes 4 and 8), the attenuating effect of BFA on μ ubiquitination (top panel) did not result from counteracting the proteasomal inhibition by ALLN, as indicated by 35S-labeled μ (middle panel), or steady-state levels of μ (bottom panel), or an effect of BFA on ubiquitination of cellular proteins in general (lanes 9–16). Similar to the situation in the 38C cells, also in the 70Z/3 cells, the effect of BFA on the ubiquitination of μ paralleled the effect of this transport blocker on the degradation of μ (Fig. 2). To conclude, only in light chain-expressing cells, where μ heavy chains were diverted to the BFA-sensitive degradation route, was the vesicular transport a prerequisite also for the ubiquitination of these heavy chains. This implies that these two transport-dependent degradation routes of light chain-assembled μ converge with the vesicular transport-independent degradation route of unassembled μ subsequent to the vesicular transport but prior to the ubiquitination of μ, eventually funneling both routes into the ubiquitin-proteasome pathway.

**DISCUSSION**

In this article we correlate the stage of B cell differentiation with two routes that lead the μ heavy chains of IgM to degradation by the ubiquitin-proteasome pathway. The first route resembles ERAD of many aberrant mammalian proteins, whose deployment to distal locations of the secretory pathway is prevented by vesicular transport-independent degradation (7–9). Interestingly, for μ heavy chains this vesicular transport-independent degradation has been observed only in light chain-deficient B cells or upon dissociation of μ heavy chains from light chains in thiol-treated B cells (2, 3, 24). Here we demonstrate this route of μ to ERAD in a developmental context. In the 70Z/3 pre-B cells, prior to expression of the conventional μ light chain, the μ heavy chains, which are either free or assembled into μ homodimers, are eliminated by the vesicular transport-independent ERAD, as concluded from the persistence of this degradation in BFA-treated or permeabilized cells. This process most likely accounts for the observation that, in pre-B cells, the majority of μ heavy chains are neither secreted nor presented at the cell surface (20–23). Nevertheless, the
Figure 5. Ubiquitination of μ is detected upon proteasome inhibition in pre-B and B cells, but it is inhibited by brefeldin A only in B cells. A, 38C cells were incubated for 4 h in the absence (−) or presence (+) of ALLN and in addition in the absence (−) or presence (+) of BFA. IgM was immunoprecipitated (IP: IgM), resolved by reducing 10% SDS-PAGE, electroblotted, and probed with a mouse anti-ubiquitin antibody followed by an HRP-conjugated anti-mouse antibody (lanes 1–4). For comparison, total proteins from cell extracts (1% of the amount used for immunoprecipitation) were resolved by reducing 7% SDS-PAGE, electroblotted, and probed with the anti-ubiquitin antibody (lanes 5–8). B, 70Z/3 cells were incubated for 18 h in the absence (−) or presence (+) of LPS, were pulse-labeled for 5 min with [35S]methionine and chased for 4 h with no additions, in the presence of ALLN, or in the presence of ALLN and BFA. IgM was immunoprecipitated (IP: IgM), resolved by reducing 10% SDS-PAGE, and electroblotted and the blot was autoradiographed (35S, middle panel). Subsequently, the blot was probed with a mouse anti-ubiquitin antibody (anti-Ub) followed by an HRP-conjugated anti-mouse antibody (top panel) and reprobed with an HRP-conjugated anti-μ antibody (bottom panel). For comparison, total proteins from cell extracts (10% of the amount used for immunoprecipitation) were resolved by reducing 10% SDS-PAGE, electroblotted, and probed with the anti-ubiquitin antibody (lanes 9–16). Arrow, the migration position of μ heavy chains as detected by anti-μ.

Efficient secretion of μS form plasma cells and the display of μM on the surface of B cells (1, 5) indicates that these μ heavy chains are neither aberrant nor transport-incompetent. Remarkably, the assembly with κ light chain enables both membrane and secretory forms of the μ heavy chain to exit the ER by vesicles and be diverted from the vesicular transport-independent ERAD. Nonetheless, although μM traverses the entire secretory pathway to be displayed at the cell surface as the mature B cell receptor, μS is transported by vesicles to degradation, as demonstrated by stabilization of μS in BFA-treated or transport-incompetent permeabilized B cells. Thus, expression of the conventional light chains, the hallmark of pre-B to B cell differentiation (20, 21), is also accompanied by diverting μ from vesicular transport-independent ERAD to vesicular transport-dependent degradation, a process that we have previously characterized for μS in B cells (2–4).

The onset of κ expression is only one of multiple changes that 70Z/3 cells undergo upon LPS stimulation, a complex process that culminates in pre-B to B cell differentiation (20–23, 28). However, the indistinguishable intracellular fate of μ heavy chains in the thiol-treated 38C B cells (3), the κ-deficient 38C-derived EH cells (2), or the NOS cells transfected with μ (34) strongly suggests that κ light chain plays a key role in this process. This notion is confirmed and extended to conventional light chains in general by our observations in 70Z/3 cells, where the λ light chain is ectopically expressed. Thus, in this context, the differentiation into B cells primarily contributes conventional light chains, which in turn affect the intracellular fate of their partner μ heavy chains. Our results clearly show that assembly of μ with either LPS-induced κ or ectopically expressed λ is tightly correlated with μ stabilization under conditions that block vesicular transport. Yet we cannot exclude B cell-specific components, other than conventional light chains, that might contribute to intracellular transport, retention, or assembly of μ heavy chains. Actually, it has been shown that transfection of κ in pre-B cells failed to fully restore the transport of the μM pre-B cell receptor complex to the cell surface (28). Likewise, we show here that the inherent capacity to promote assembly improves upon differentiation from pre-B to B cells, as judged by the increased assembly of μ with the ectopically expressed λ upon LPS stimulation. This improvement occurs despite the onset expression of the endogenous κ light chain, which is shown here to preferentially assemble with μ. Most importantly, ectopic light chain expression does not trigger endogenous light chain expression. It argues that conventional light chains, rather than other B cell-specific components, play a key role in diverting the route of μ heavy chains in the course of pre-B to B cell differentiation.

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The indistinguishable intracellular fate of μ that is assembled with either κ or λ stands in contrast with the effects exerted by the SLC. Both SLC and κ interact with μ through its Cμ1 domain, which, because of its stable interaction with BiP (29–33), probably directs free μ or μβ homodimers to the vesicular transport-independent ERAD. Therefore, it is anticipated that either SLC or κ would equally counteract Cμ1-mediated retention and displace BiP. Indeed, μS assembled with SLC is secreted from 70Z/3 cells, provided that its additional retention signal in the μS tail piece (μStpCys) is neutralized (34). Moreover, μM associated with SLC, Igκ, and Igβ forms the pre-B cell receptor visualized on the surface of several pre-B cell lines (28, 35–37), where it plays a critical role in development of B-lymphocyte lineage (22, 23, 34). Yet we could detect only slight maturation of μM in the unstimulated 70Z/3 pre-B cells expressing either endogenous SLC or λ ectopically. Similarly, only after enrichment could Brons et al. (28) detect a minute fraction of lectin-bound transported μM in NALM-6 pre-B cells. Thus, the assembly of μ with either SLC or κ may reflect a quantitative difference between the relative amounts of these chains. Indeed, very low levels of μ-SLC hemimers are detected in naive 70Z/3 cells, and μ remains bound to BiP mostly as free μ or μβ homodimers. Hence, if these minute amounts of μ-SLC complexes could exit the ER, they would hardly affect the mode of μS degradation or the extent of BiP association, and mature μM would be just at the lowest level of detection. Thus, one
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The involvement of the proteasome in the degradation of Ig heavy chains has been reported only for unassembled chains such as nonsecreted κ light chain (38), mutant light chains (39), membrane γ2b heavy chain (40), and μ heavy chain (24). In this study we show that degradation of unassembled μ in light chain-negative cells, and also of assembled μ in cells expressing conventional light chains, involves the proteasome as the proteolytic system and ubiquitin as the tagging mechanism. The unassembled or disassembled μ heavy chains resemble other mammalian ERAD substrates, because they are targeted to degradation independently of vesicular transport. On the other hand, upon assembly with either κ or λ, μ is diverted to a BFA-sensitive, vesicular transport-dependent degradation process. Nonetheless, these two distinct routes to degradation converge at the ubiquitin-proteasome pathway. This is demonstrated by the marked stabilization and accumulation as polyubiquitinated species of both unassembled and assembled μ in light chain-negative and κ-expressing cells, respectively, provided the proteasome is blocked.

Our data clearly show that vesicular transport is an obligatory step that is a prerequisite for ubiquitination of μ only in κ-expressing cells. Although the function of this vesicular transport remains to be elucidated, recent studies in yeast demonstrate that vesicular transport-dependent degradation is neither restricted to μ heavy chain nor to B-lymphotyes. Genetic data show that degradation of luminal ERAD substrates such as CPY* and PrA* requires vesicular trafficking between the ER and Golgi (41–43). The ubiquitination and elimination of μ by the cytosol-oriented components of the ubiquitin-proteasome pathway indicates that μ must dislocate from the ER lumen back to the cytosol. In κ-expressing cells, μ should be transported from the ER by vesicles prior to its dislocation, ubiquitination, and degradation. Hence, μ may dislocate to the cytosol from a distal compartment. If μ is handled like other ERAD substrates and its dislocation is mediated by the Sec61p translocon (44–47), it may dislocate through the Sec61p located in ER-Golgi intermediate compartment (48). Involvement of compartments distal to the ER in quality control is suggested by immunolocalization to the Golgi apparatus and pre-Golgi intermediates of deglucosylating endomannosidase, UDP-glucose:glycoprotein glucosyltransferase, glucosidase II, and calreticulin (49, 50), key components of the protein quality control machinery.

Alternatively, dislocation to the cytosol may take place from the ER, provided that the degradation substrate is retrograde-transported to this compartment. Again, this mechanism is supported by genetic data from yeast that demonstrate anterograde and retrograde vesicular trafficking in the ER-Golgi system as a prerequisite for the degradation of CPY* and PrA*, two well established luminal ERAD substrates (41, 42). Nevertheless, even if the retrograde-transported substrate is dislocated from the ER, its elimination is not necessarily executed through ERAD. A quality control mechanism that is independent of HRD/DER has been reported in yeast (51). Recently, this HRD/DER-independent proteasomal degradation, designated HIP, was shown to depend on vesicular transport and to involve Usp5p as E3 and possibly Ubp4p and Ube5p as E2 (43). It has been suggested that saturation of the vesicular transport-independent ERAD machinery is responsible for diverting overexpressed quality control substrates (e.g. CPY*) to this vesicular transport-dependent HIP pathway (43). This situation, however, does not apply to the μ heavy chain in B cells, where it is the assembly with conventional light chains that diverts μ to the transport-dependent pathway. Interestingly, a very recent report from yeast suggests that the Sar1p/COPII machinery is required for sequestration of the membrane ERAD substrate CFY into ER subdomains before its proteasomal degradation, but this function is independent of the role of Sar1p/COPII machinery in ER-Golgi vesicular transport (52).

The developmental transition, from the vesicular transport-independent route to ERAD of unassembled μ heavy chains in pre-B cells to the vesicular transport-dependent route to degradation by the proteasome-ubiquitin pathway of μ in κ-expressing B cells, adds new perspective to the post-translational regulation of the immune response and the differentiation of lymphocytes of the B lineage. Moreover, our study extends the degradation along the secretory pathway beyond the quality control elimination of aberrant proteins. In response to developmental cues, two routes lead proteins selected by their assembly state to the ubiquitin-proteasome pathway.
Two Routes Lead to IgM Degradation

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