Reduction of Interchain Disulfide Bonds Precedes the Dislocation of Ig-μ Chains from the Endoplasmic Reticulum to the Cytosol for Proteasomal Degradation*

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Proteins that fail to fold or assemble in the endoplasmic reticulum (ER) are generally dislocated across the membrane to be degraded by cytosolic proteasomes. To investigate how the quality control machinery handles individual subunits that are part of covalent oligomers, we have analyzed the fate of transport-competent Ig light (L) chains that form disulfide bonds with short-lived μ heavy chains. When expressed alone, L chains are secreted. In cells producing excess μH9262/H9251 peptide their correct three-dimensional structure are retained in the folding machinery (1), ensuring that proteins that fail to attain the native conformation are degraded by proteasomes, L chains are stable. Few L chains are secreted; most reassociate with newly synthesized μ chains. Therefore, interchain disulfide bonds are reduced in the ER lumen before the dislocation of μ chains in a site from which freed L chains can be rapidly reinserted in the assembly line. The ER can thus maintain the simultaneous formation and reduction of disulfide bonds.

The endoplasmic reticulum (ER) is the port of entry for proteins destined to the exocytic pathway. In this organelle, proteins fold and assemble under the assistance of a vast array of chaperones and enzymes (1). As many membrane or secretory proteins contain disulfide bonds that are essential to attain the native conformation, a central role is played by ER-resident oxido-reductases, which transfer oxidative equivalents to nascent proteins (2). Therefore, cascades of specific protein-protein interactions are required to co-ordinate the oxidation and isomerization reactions occurring in the ER (3–5).

A stringent quality control system is coupled to the ER folding machinery (1), ensuring that proteins that fail to attain their correct three-dimensional structure are retained in the ER and eventually degraded after a lag after synthesis that varies among individual substrates (6, 7). We have recently shown that the processing of N-linked glycans times the degradation of two glycosylated Ig subunits, μ and J chains (8), allowing the discrimination between newly translocated and terminally unfolded molecules. Substrates of ER-associated degradation are dislocated across the ER membrane through Sec61 to be degraded by proteasomes (9–12).

Little is known on the mechanisms that gate the dislocation channels and determine the directionality of transport across the ER membrane (13–15). An important issue is to what extent substrates must be unfolded to negotiate dislocation. While the Sec61 channel may undergo some lateral expansion (16), it is unlikely that large oligomeric proteins can be transported across the ER membrane without perturbing the ionic gradients that exist between the ER lumen and the cytosol. We have chosen immunoglobulins (Igs) as model systems to investigate how the quality control machinery handles oligomeric complexes in which individual subunits have different half-lives. Ig light (L) chains can be secreted also when not paired to heavy (H) chains (17). In contrast, unassembled H chains are retained in the ER because of the fact that their CH1 domains bind tightly to BiP (18). Proteasomes are involved in the degradation of unassembled H chains in myeloma cells (14, 15, 19).

An interesting feature of secretory IgM is that their quality control varies during B cell development, determining the ultimate fate of the molecule (for review, see Ref. 20). IgM is secreted by plasma cells only as pentamers containing a J chain or hexamers (20). This is due to the fact that the carboxyl-terminal cysteine of secretory μ (μs) chains (Cys-575) serves as a three-way switch mediating the assembly, retention, and degradation of unimerized IgM subunits (21–23). For reasons that are still unclear, IgM polymerization is inefficient in B cells (24). Therefore, most μs chains are retained and eventually degraded despite assembling with L chains to form μs2L2 complexes (25–28). In contrast, analogous complexes containing membrane μ chains (μm2L2) negotiate assembly with signaling components (Ig-α and Ig-β) and are transported to the cell surface of B lymphocytes (29, 30) where they act as antigen receptors.

Because of the intrinsic complexity of IgM intracellular traffic in B cells and the unavailability of antibodies efficiently discriminating μm and μs, we decided to first investigate the handling of covalent complexes containing subunits with different half-lives in a simpler model system. Suitably engineered Ig H and L chains were thus expressed in myeloma cells lacking endogenous Igs. A chimeric μ chain carboxyl-terminally extended with the transmembrane region of the T cell receptor α chain (μ-TCRα) was selected because it is degraded by proteasomes even when assembled with λ light chains (22). Since normal and malignant plasma cells synthesize L chains

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The abbreviations used are: ER, endoplasmic reticulum; Endo H, endoglycosidase H; Ig, immunoglobulin; H, heavy; L, light; TCRα, T cell receptor α chain; NP, (4-hydroxy-3-nitrophenylacetyl); PNGase F, peptide N-glycosidase; μs, secretory μ; μm, membrane μ; ZL2,H, N’-benzoxycarbonyl-Leu-Leu-Leu-aldehyde.

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in excess, it has been difficult to analyze the fate of λ chains that partner up with short-lived μ-TCRαs in J558L cells. To circumvent this problem, we generated myeloma transfectants expressing more μ-TCRαs than λ chains. In this line, almost all “innocent” λ chains are assembled with short-lived μ. Our results demonstrate that covalent H-L and H2L2 complexes are dissociated prior to the dislocation of μ-TCRα chains to the cytosol. Most freed λ chains reassemble with μ-TCRα, and only a few are secreted. Secretory IgM in B cells shares a similar fate. These results suggest that interchain disulfide bonds are reduced in a site from which short- and long-lived subunits can be efficiently sorted. The former proceed to dislocation, while the latter are reinserted into the assembly line or exported to the Golgi to be secreted.

**EXPERIMENTAL PROCEDURES**

*Cell Lines, Transfectants, Antibodies, and Reagents*—NSO myeloma cells were stably transfected as described previously (8, 22) with plasmids driving the expression of μ-TCRαs. The entire murine λ light chain coding sequence was excised from pREP10A.1 (20) and inserted into pcDNA3.1A. Transfectants were selected on hygromycin. Upon assembly, μ-TCRα and λ chains generate binding sites specific for the hapten NP (22, 31).

The B lymphoma WEHI-231 or its variant WEHI-231-J3E5 (28) expressing murine J chains (W.231-J) were kind gifts of Dr. Ron Corley (Boston University). Transfectants were maintained in Dulbecco’s modifed Eagle’s medium or RPMI medium supplemented with the appropriate selection drug as described previously (14, 22). The antibodies and reagents used have been described previously (8). Pulse-Chase Assays, Immunoprecipitation, and Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis—Pulse-chase assays, immunoprecipitations, and Western blotting were performed as described previously (8, 22). For sequential immunoprecipitation, samples were first incubated with either rabbit anti-mouse μ antibodies followed by protein A-Sepharose or NP-Sepharose. Immunoprecipitation was repeated once with the first reagent to ensure complete capture of the reactive proteins. The supernatants were then incubated with the second reagent (anti-L or anti-μ, respectively). Protein G-Sepharose beads were used with goat anti-λ. Immunoprecipitates were treated with or without endoglycosidase H (Endo H, Roche Molecular Biochemicals) or PNGase F (New England Biolabs, Beverly, MA) as recommended by the suppliers. Fluorograms were scanned, and the relevant bands were quantified by the ImageQuant software (Molecular Dynamics, Sunnyvale, CA).

**RESULTS**

Generation and Characterization of Myeloma Transfectants Expressing Different Amounts of μ-TCRα and λ Chains—Assembly of H with L chains normally results in Ig secretion (Ref. 20 and references therein). However, chimeric μ chains extended with the transmembrane region of the TCRα chain, which contains a dominant ER-associated degradation targeting signal (32), are rapidly degraded even when covalently assembled with λ (Refs. 8 and 22 and see below).

To analyze the fate of transport-competent λ chains that form covalent complexes with these short-lived chimeric μ chains, we generated NSO transfectants expressing μ-TCRα, λ, or both chains in different amounts (Fig. 1). A clone producing μ-TCRα in excess (N[μ>λ], Fig. 1, lane 1) was selected. In these cells, bands corresponding to monomeric and dimeric μ-TCRs but very few free λ chains were detectable under nonreducing conditions (Fig. 1, lane 5). Most λ chains are part of covalent μ-TCRα and μ-TCRα,λ complexes.

Assembly with μ-TCRα Retards the Secretion of λ Chains—While unassembled μ-TCRα chains were not secreted (Fig. 2, lanes 1–3), about half of the λ chains synthesized during a 5-min pulse could be detected after 4 h of chase in the supernatants of NSO transfectants lacking H chains (Fig. 2, λ, lanes 4–6). The synthesis of μ-TCRα chains dramatically reduced λ secretion by the N[μ>λ] transfectant (Fig. 2, lanes 7–9), and less than 10% of λ were detected extracellularly at the end of the chase. Also in these cells, μ-TCRα chains were not secreted (Fig. 2, lane 9). However, while μ-TCRα was extensively degraded, the majority of λ chains was recovered in the lysates of cells chased for 4 h (Fig. 2, lane 8). Thus, although in this transfectant most λ chains are covalently assembled with

**FIG. 1.** Characterization of myeloma transfectants expressing λ and/or μ-TCRα chains. NSO cells were transfected with plasmids driving the expression of μ-TCRα (lanes 3 and 7), λ (lanes 4 and 8), or both in different amounts (lanes 1, 2, 5, and 6). A clone expressing μ-TCRα in excess (NSO[μ>λ], lanes 1 and 4) was selected for further studies. Lysates were resolved on a gradient gel (3–14% acrylamide) under reducing (lanes 1–4) or nonreducing (lanes 5–8) conditions, transferred to nitrocellulose filters, and decorated with anti-μ or anti-λ antibodies (upper and lower parts of the gels, respectively; see horizontal lines). Arrows on the right-hand margin indicate the mobility of different subunits and assemblies identified by their electrophoretic mobility and serological reactivity. WB, Western blotting.

**FIG. 2.** Assembly with short-lived μ-TCRα prevents the secretion of λ chains without inducing their degradation. After a 5-min pulse, N[μ-TCRα] (lanes 1–3), N[λ] (lanes 4–6), or N[μ>λ] (lanes 7–9) cells were chased for 4 h. Cell lysates (C) and supernatants (S) were immunoprecipitated with anti-μ (top panel) or anti-λ (bottom panel) antibodies and resolved under reducing conditions. Closed and open arrows on the left-hand margin point to μ and λ chains, respectively.
The reduction of ER-associated degradation substrates in the ER

Interchain H-L disulfide bonds are reduced before the dislocation of \( \mu \)-TCR\( \alpha \) chains. Pulse-labeled N[\( \mu \geq \lambda \)] cells were chased for the indicated times before lysis and immunoprecipitation with different antibodies. A, anti-\( \mu \) immunoprecipitates were resolved under reducing conditions. B, the intensity of the \( \mu \)-TCR\( \alpha \) and \( \lambda \) chain bands was quantified by densitometry (average of two experiments). C, lysates were first incubated with NP-Sepharose (NP-Sep) beads (top panel) to capture \( \mu \)-TCR\( \alpha \) assembled with \( \lambda \) followed by anti-\( \mu \) (bottom panel) to bring down free \( \mu \)-TCR\( \alpha \) chains. D, densitometric quantification, performed as in A, revealed different half-lives of assembled and free \( \mu \)-TCR\( \alpha \) chains.

\( \mu \)-TCR\( \alpha \) (see Fig. 1), the two subunits appeared to have different fates.

\( \mu \)-TCR\( \alpha \) and \( \lambda \) Chains Present in Covalent Complexes Have Different Fates—More detailed pulse-chase assays were performed to investigate the fate of individual Ig subunits in NSO[\( \mu \geq \lambda \)] cells (Fig. 3). Lysates were immunoprecipitated with anti-\( \mu \) antibodies to capture all \( \mu \)-TCR\( \alpha \) and the fraction of \( \lambda \) chains associated to them (Fig. 3A). After a lag, \( \mu \)-TCR\( \alpha \) chains were degraded. On the contrary, the intensity of the \( \lambda \) chain band increased during the initial chase period, reflecting progressive assembly of newly synthesized \( \lambda \) with \( \mu \)-TCR\( \alpha \), and remained almost constant later in the chase when the degradation of radioactive \( \mu \)-TCR\( \alpha \) became evident (Fig. 3, A and B). Analysis of the samples under nonreducing conditions (not shown) confirmed that assembly between \( \lambda \) and \( \mu \)-TCR\( \alpha \) was stabilized by interchain disulfide bonds (Fig. 1). These results suggested that \( \lambda \) chains that were initially disulfide-bonded to radioactive \( \mu \)-TCR\( \alpha \) reassociated with cold \( \mu \)-TCR\( \alpha \) chains synthesized during the chase, implying that interchain disulfide bonds are continuously reduced and reformed in the ER.

Assembly with \( \lambda \) Chains Retards the Degradation of \( \mu \)-TCR\( \alpha \)—We have previously shown that the degradation of two transport-competent \( \mu \) chains, \( \mu_{n} \) and \( \mu_{ACH1} \), is slower in J558L (which produces \( \lambda \) chains in excess) than in NSO cells (31, 33), suggesting that assembly with \( \lambda \) chains can protect \( \mu \) chains from degradation. The N[\( \mu \geq \lambda \)] transfectant synthesizing \( \lambda \) chains in excess allowed us to compare the fate of free and assembled \( \mu \)-TCR\( \alpha \) chains in the same cell. To this end, lysates of pulse-chased cells were first incubated with NP-Sepharose beads, which bind only properly assembled V\( \gamma \)-V\( \lambda \) (31). The leftovers were then incubated with anti-\( \mu \) antibodies to precipitate unassembled \( \mu \)-TCR\( \alpha \) chains. As described above for \( \lambda \) chains (Fig. 3A), the fraction of radioactive \( \mu \)-TCR\( \alpha \) chains precipitating with NP-Sepharose increased in the first chase point, reflecting assembly of newly made \( \lambda \) chains into hapten-binding complexes (Fig. 3C, upper panel). After 1 h of chase, equal amounts of radioactive \( \mu \)-TCR\( \alpha \) chains were present in the assembled and free pools (Fig. 3C, lane 3). During further chase, the intensity of the NP-precipitable band decreased more slowly, suggesting that assembly with \( \lambda \) retarded their degradation (Fig. 3D).

Reduction of Interchain Disulfide Bonds in the ER Is Coupled to Dislocation—Next we asked whether the reduction of interchain disulfide bonds in the ER lumen is coupled to dislocation. We have previously shown that the dislocation of unassembled \( \mu \) chains is coupled to degradation (14). Likewise, \( \mu \)-TCR\( \alpha \) chains were found in the microsomal pellet after incubation with proteasome inhibitors (Fig. 4A) when deglycosylated J chains were detectable in the cytosol (14). Therefore, although the degradation of \( \mu \)-TCR\( \alpha \) chains is insensitive to ER mannosidase I inhibitors (8) their dislocation requires active proteasomes.

As expected (8), proteasome inhibitors prevented the degradation of \( \mu \)-TCR\( \alpha \) chains in N[\( \mu \geq \lambda \)] cells (Fig. 4B, compare lanes 2 and 3 in the top panel). Some \( \lambda \) chains were stabilized as well (Fig. 4B, middle panel) but to a much smaller extent than \( \mu \)-TCR\( \alpha \). The stabilization was observed also in cells expressing only \( \lambda \) chains (Fig. 4B, bottom panel), suggesting that some unassembled wild type \( \lambda \) chains are degraded by proteasomes (17, 34, 35). The increase in the intracellular \( \lambda \) pool was not due to inhibition of secretion by ZL\( \alpha \)H since similar amounts of \( \lambda \) could be recovered in the supernatants (not shown). It is noteworthy that, when the dislocation of \( \mu \)-TCR\( \alpha \) was prevented by blocking proteasome activity, the amount of unassembled \( \lambda \) did not increase significantly and covalent \( \mu \)-TCR\( \alpha \)-\( \lambda \) complexes accumulated (Fig. 4C, lanes 3 and 6). Taken together, these findings suggested that the reduction of the interchain disulfide bonds is coupled with dislocation.

Proteasomal Degradation of Unpolymerized Secretory IgM in B Cells—Having shown that L chains are dissociated in the ER lumen prior to the dislocation of \( \mu \)-TCR\( \alpha \) chains in myeloma transfectants, we analyzed whether this mechanism is important also in B lymphocytes. These cells retain most \( \mu \) chains in the ER but express \( \mu_{m} \) chains on the surface as antigen receptors (21, 25, 29, 30).

The differential trafficking of \( \mu_{n} \) or \( \mu_{m} \) can be studied in W.231-J B lymphoma cells, which produce similar amounts of the two isoforms (Fig. 5). Despite \( \mu_{n} \) being 22 residues longer than \( \mu_{m} \) (36), the presence of an additional N-glycan in the \( \mu_{n} \)-carboxy-terminal tailpiece makes it difficult to distinguish the two chains electrophoretically (Fig. 5A, lane 1). To allow unambiguous identification of \( \mu_{n} \) and \( \mu_{m} \), samples were thus treated with PNGase F, an enzyme that removes all \( N \)-glycans (28). Proteasome inhibitors did not...
some inhibitors induced the accumulation of Endo H-sensitive chains (Fig. 5C, compare lanes 4 and 6). It is not easy to establish the relative amounts of $\mu_m$ and $\mu_s$ present in this band; however, the results obtained with PNGase F suggest that $\mu_s$ predominated. Mature $\mu_m$ chains were easily detectable after 4 h of chase as an Endo H-resistant band of about 82 kDa (Fig. 5, A–C, tailed arrows). The abundance of this species was not altered by proteasome inhibitors (Fig. 5, A, lanes 5 and 7; B, lanes 2 and 3; and C, lanes 3–6). However, we often detected more $\mu_m$ in cells chased with proteasome inhibitors, indicating that some $\mu_m$ are degraded by proteasomes as well probably due to their inefficient assembly with Ig-α and/or Ig-β.

Electrophoresis under nonreducing conditions (Fig. 5C, lanes 7–9) revealed that, while some free $\mu$ chains were detectable at the end of a 5-min pulse, both $\mu_m$ and $\mu_s$ were associated covalently with K chains at the end of the chase. The presence of proteasome inhibitors induced the accumulation of a band of about 200 kDa, likely consisting of $\mu_s$2L2 complexes. Some high molecular weight aggregates were also seen (Fig. 5C, arrows).

Noteworthy is that the intensity of the K chain band was only slightly increased in cells treated with proteasome inhibitors (Fig. 5C, compare lanes 3–6). This observation suggested that, like in the NSO transfectants, radioactive K chains that were part of covalent complexes with short-lived $\mu_s$ chains were dissociated and recycled also in B cells. To confirm this, the anti-$\mu$ immunoprecipitates from a pulse-chase assay were resolved under reducing conditions, and the intensity of $\mu$ and K chain bands was quantified by densitometry (Fig. 5D). Clearly, radioactive K chains appeared to be more stable than Endo H-sensitive $\mu_s$ chains. Western blot and immunoprecipitation assays (not shown) confirmed that in W.231-J cells used in these studies the vast majority of K chains was covalently assembled to $\mu$ chains.

**DISCUSSION**

Translocating a polypeptide across a membrane without perturbing ionic gradients is a formidable task for living cells. It has been clearly established that proteins must be unfolded to penetrate mitochondrial membrane (38). Similarly the post-translational entry of polypeptides into the ER of yeast cells requires unfolding (39). So far little is known about whether, and to what extent, unfolding is necessary for luminal proteins to be dislocated across the ER membrane for proteasomal destruction. We have exploited Ig subunits with different half-lives to determine whether disulfide bonds are reduced in the ER lumen before dislocation. In the two experimental systems used, nonsecreting B cell lymphomas and suitably engineered myeloma transfectants, transport-competent L chains assemble covalently with short-lived $\mu$ chains.

Our results allow the following main conclusions to be drawn. First, in B lymphoma cells $\mu_s$ chains are degraded by cytosolic proteasomes. Second, H-L interchain disulfide bonds are reduced prior to the dislocation of short-lived $\mu_s$ chains. Third, most released L chains reassemble with newly made Ig chains.

**Degradation of Secretory IgM in B Lymphoma Cells**—Because of the inefficiency of IgM polymerization in B cells, the $\mu_s$ Cy5-575 residues exert their function mainly as a retention and degradation targeting element (32). As a result, $\mu_s$ “hemimers” and $\mu_s$2L2 “monomers” accumulate in the ER and are eventually degraded. The observation that lactacystin, ZL3H, carboxybenzyl-Leu-Leu-Leu-vinylsulfone, and N-acetyl-Leu-Leu-norleucinal (40) prevent the degradation of $\mu_s$ chains indicates the involvement of cytosolic proteasomes. $\mu_s$ chains are degraded also in a W.231 transfectant expressing J chains.

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2 J. Haimovich and E. Rabinovich, personal communication.
**Reduction of ER-associated Degradation Substrates in the ER**

Few IgM pentamers and hexamers are secreted by these cells (28) confirming that, while allowing the production of pentamers, the synthesis of J chains is not sufficient to initiate efficient IgM polymerization (20). Also some \( \mu_s \) chains are degraded by proteasomes, probably reflecting inefficient assembly with Ig-\( \alpha \) and Ig-\( \beta \).

Deglycosylated J chains become clearly detectable when proteasomes are inhibited, implying that a few J chains are dislocated by cytosolic N-glycanases (Ref. 14 and references therein). In contrast, deglycosylated \( \mu_m \) chains are not detectable under the same conditions, suggesting that as myeloma cells degradation and dislocation are coupled events.

As described previously for unassembled \( \mu_m \) and \( \mu_s \) chains in myeloma cells (8), kifunensine inhibited \( \mu_s \) degradation in W231-J cells indicating that N-glycan processing is important for timing IgM quality control also in B lymphomas. During the time allocated before expulsion from the ER, both \( \mu_m \) and \( \mu_s \) fold and assemble with L chains, forming intra- and interchain disulfide bonds.

**Reduction of H-L Interchain Disulfide Bonds in the ER Lumen**—Once the \( \mu_s \) N-glycans undergo processing by kifunensine-sensitive mannosidases, the \( \mu_s \) present in monomers and hemimers are targeted for dislocation. How are these covalent complexes handled? Our findings indicate that the interchain disulfide bonds that link L chains to short-lived H chains are reduced prior to the dislocation of the latter, that is in the ER lumen. In myeloma cells engineered to produce short-lived \( \mu \)-TCR\( \alpha \) chains in excess, most \( \lambda \) are assembled covalently to \( \mu \)-TCR\( \alpha \). Yet \( \lambda \) chains are stable, implying that they are dissociated from \( \mu \)-TCR\( \alpha \) before these are degraded by proteasomes. Our pulse-chase assays indicate that dissociation occurs before, and seems to be coupled to, the dislocation step. The majority of freed \( \lambda \) chains reassemble with cold \( \mu \)-TCR\( \alpha \) chains synthesized during the chase. Thus, the dissociation of H and L chains occurs in a site from which released L chains can rapidly gain access to the assembly line. Only a few L chains can escape this Sisyphean effort to make antibodies with the wrong partner chain and are secreted (Fig. 6). The ER

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**FIG. 5.** *Proteasomal degradation of \( \mu_s \) chains in B lymphoma cells is preceded by reduction of H-L interchain disulfide bonds.* W.231-J cells were pulsed for 5 min and chased for different time periods with or without proteasome or mannosidase inhibitors as indicated in each panel. A, preferential degradation of \( \mu_s \) chains. Anti-\( \mu \) immunoprecipitates were treated with or without PNGase F to remove all N-glycans. The mobility of deglycosylated \( \mu_m \) and \( \mu_s \) is indicated by arrows on the right-hand margin. Mature Endo H-resistant \( \mu_m \) chains are detectable in cells chased in the presence or absence of ZL\( \mu \)-H (Z) (lanes 5 and 7, tailed arrow). B, mannose trimming times the degradation of \( \mu_s \) chains in B lymphoma cells. Anti-\( \mu \) immunoprecipitates were treated with or without PNGase F before electrophoresis under reducing conditions. Exogenous J chains were also degraded by proteasomes in these cells. A deglycosylated J chain was clearly detectable in ZL\( \mu \)-H (Z)-treated cells. Kifunensine (K) inhibited also in part the dislocation and the degradation of exogenous J chains (not shown). The tailed arrow points at mature \( \mu_m \) chains. C, short-lived \( \mu_s \) chains accumulating in cells treated with proteasome inhibitors are sensitive to Endo H and covalently assembled with K chains. Anti-\( \mu \) immuno-precipitates were treated with or without Endo H to selectively remove immature N-glycans. Samples were resolved on a standard 10% acrylamide gel under reducing conditions (lanes 1–6) or on a gradient gel (2–10% acrylamide) under nonreducing conditions (lanes 7–9). Note that radioactive K chains (k) precipitated by anti-\( \mu \) do not decrease as much as \( \mu \) chains. The tailed arrow points to mature Endo H-resistant \( \mu_m \) chains (lanes 3–6). D, recycling of K chains in B lymphomas. Densitometric quantification of the \( \mu_m \) and K (k) chain bands present in the anti-\( \mu \) immunoprecipitates from pulse-chased W.231-J cells.

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**FIG. 6.** *Formation and reduction of interchain disulfide bonds in the ER.* Soon after synthesis, \( \lambda \) chains form interchain disulfide (SS) bonds with short-lived \( \mu \)-TCR\( \alpha \) chains. Covalent \( \mu \)-TCR\( \alpha \)-\( \lambda \) complexes are dissociated prior to dislocation of \( \mu \)-TCR\( \alpha \), releasing \( \lambda \) chains that are rapidly reassembled with newly made \( \mu \)-TCR\( \alpha \) and are in small part secreted.
seems to be the best candidate to host this chain of events. However, it is also possible that the H-L dissociation that precedes μ dislocation occurs, at least in part, in the intermediate compartment, which has been shown to contain some Sec61 channels (41). L chains released in the ER-to-Golgi intermediate compartment might be secreted. The results shown in Fig. 5 suggest that L chains are sorted from short-lived μ chains and recycled also in B lymphoma cells.

In agreement with previous findings in both myeloma and B lymphoma transfectants (26, 31, 33), assembly with L chains and recycling also in B lymphoma cells.

Redox Control in the ER—It is generally accepted that the redox conditions in the ER are more oxidizing than those in the cytosol (3, 4, 43). The observation that interchain disulfide bonds are reduced before dislocation of short-lived μ chains implies that molecules endowed with reductase activity be present in the vicinity of active dislocans. The identity of these molecules and the mechanisms that maintain them in the reduced state within the oxidizing milieu of the ER are unknown. Reduced protein disulfide isomerase has been shown capable of dissociating cholera toxin, releasing A chains that can parasitize the ER-associated degradation dislocation pathways to reach their targets in the cytosol (44). As oxidized protein disulfide isomerase is known to bind nascent proteins to facilitate disulfide bond formation (45), the question arises of if and how reduced protein disulfide isomerase can be generated in the vicinity of active dislocans. In mammalian cells, Ero1-L α and β oxidize protein disulfide isomerase4 and may play a key role in controlling the balance between formation and reduction of disulfide bonds in the ER. It is also possible that other ER oxidoreductases, such as Erp72, Erp57, or p5, whose redox state does not seem to depend on the activity of Ero1-L α and β4 are involved in breaking the H-L interchain bonds for μ chain degradation.

Whatever the molecular mechanism involved, our data imply that the redox state in the ER must be specifically and precisely controlled to allow the concomitant formation and reduction of disulfide bonds within the same compartment (3–5). It will be of interest to determine the molecules involved and the mechanisms underlying their precise localization within the ER.

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