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Interaction of BiP with newly synthesized immunoglobulin light chain molecules: cycles of sequential binding and release

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Here we show that not only transport defective but all immunoglobulin light chains interact with BiP. Association of BiP with its ligand takes place during or shortly after translation of the light chains. The biological half life of the BiP–light chain complex depends on the fate of the light chains. Light chains which are secreted interact with BiP for only a very short time. In contrast, the complex is biologically more stable in cells which do not secrete their L chains. In these cells, dissociation from BiP correlates with the biological half life of the L chains arguing for a degradation pathway in the endoplasmic reticulum. Instead of being degraded in association with its ligand, BiP is released from the complex and binds to newly synthesized polypeptides. These results support the notion that both H and L chains require the chaperoning function of BiP before or during the process of antibody assembly.

Key words: BiP/chaperone/intracellular degradation/secretion/subunit assembly

Introduction

BiP was originally described as a protein which associates with immunoglobulin (Ig) heavy (H) chains if those are not bound to light (L) chains (Haas and Wabl, 1983). A variety of additional ligands has meanwhile been described (Gething *et al.* 1986; Dorner *et al.*, 1987; Kassenbrock *et al.*, 1988; Ng *et al.*, 1989; Machamer *et al.*, 1990; Blount and Merlie, 1991; Suzuki *et al.*, 1991), the majority of which can be formally divided into two groups. One type of ligand, usually a subunit of a multimeric protein, interacts only briefly with BiP. The other group comprises polypeptides which are unable to undergo post-translational modifications or oligomerization required to gain their native conformation. Such ligands associate with BiP in a more stable fashion and accumulate in the endoplasmic reticulum (ER). These two types of interaction are referred to as 'transient' or 'permanent' respectively.

The permanent type of BiP ligands can further be subdivided into two groups. One group usually comprises malfolded structures unable to participate in the formation of functional proteins. These polypeptides remain in the ER and may enter the novel degradation pathway recently described (Klausner and Sitia, 1990). In the case of transient interactions, ligands may further assemble with either themselves or other subunits into multimeric proteins which are subsequently exported from the ER.

The other type of permanent BiP ligand can become transient. It has been shown that H chains which were previously permanently bound to BiP are indeed able to participate in the formation of complete antibody molecules (Hendershot, 1990). Thus, if there is no general structural defect, BiP associated molecules of the permanent type may not be malfolded *per se* but only lack some component required for continuing the normal folding pathway.

Usually, Ig L chains can be secreted in the absence of H chains (Baumal *et al.*, 1973). In a few exceptional cases, however, export from the ER is dependent on association with H chain (Köhler *et al.*, 1976; Oi *et al.* 1983). Recently, it has been reported that such L chains are bound to BiP (Nakaki *et al.*, 1989; Dul and Argon, 1990; Ma *et al.*, 1990). These L chains do not belong to the 'malfolded' type of BiP ligands since they are assembled into antibody molecules when H chains are co-expressed. This fact raised the possibility that BiP binding to L chains is a general event forming stable complexes in cells which do not secrete their L chains and transient complexes in those which do. The reported inability to detect BiP–L chain interaction in L chain secreting cells would thus merely be the result of experimental conditions inappropriate to detect transient BiP–ligand interactions.

Our results demonstrate that the occurrence of BiP–L chain heterodimers is independent of the later fate of the BiP ligand. The interaction between the two proteins is of the 'permanent' type with non-secreted L chains and of the 'transient' type in the case of secreted L chains.

Results

'Permanent' interaction of BiP with L chains in non-secreting NS1 cells

In order to study the nature of BiP interaction with Ig L chains that are not secreted, we analysed the murine myeloma cell line NS1 which produces but does not secrete κ L chains (Köhler *et al.*, 1976). BiP binding to these L chains has been described (Nakaki *et al.*, 1989; Ma *et al.*, 1990). Cells were pulse labelled for 30 min and chased for different times after which cell lysates or equivalent amounts of cell culture supernatant were subjected to immunoprecipitation.

When L chains were precipitated, a time dependent decrease in the amount of radiolabelled L chains was seen (Figure 1a). As expected, nearly no labelled L chain was recovered from the cell culture supernatant (Figure 1a), confirming that these cells did not secrete their L chains. Thus, the time dependent decrease in labelled L chains reflected intracellular degradation of the polypeptide chains which in this experiment occurred with a half time of ~50 min (Figure 1b). The slight increase in the intensity of the L chain band noted at the 10 min chase point was the result of an experimental artefact and not due to residual incorporation of [^{35}S]methionine after chase initiation (Sitia *et al.*, 1987; Braakman *et al.*, 1991).

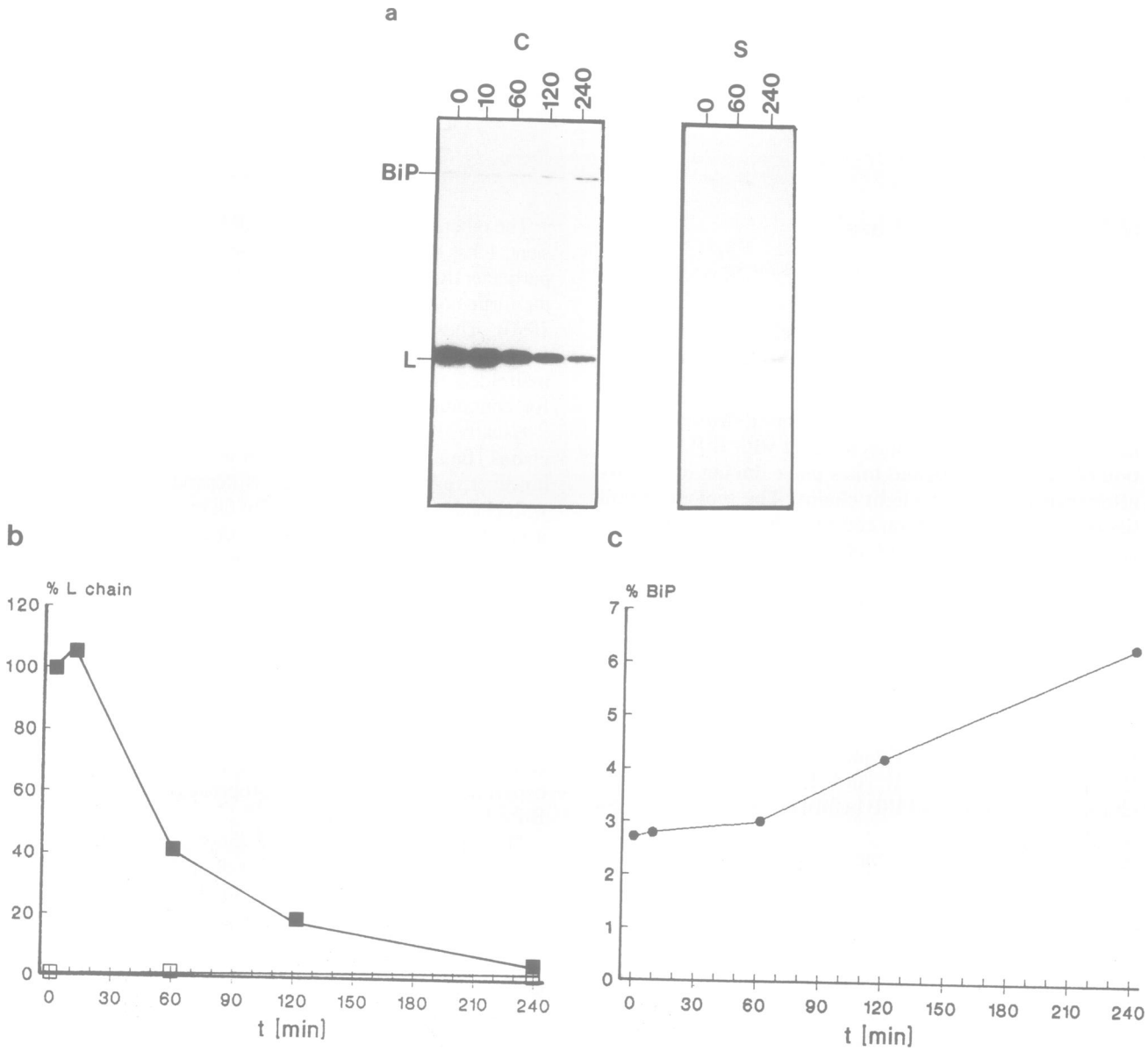

Fig. 1. Pulse–chase analysis of the interaction of BiP with non-secreted L chains in NS1 cells. NS1 cells were pulse labelled for 30 min with [^{35}S]methionine and chased for 0, 10, 60, 120 and 240 min as indicated. Cell lysates (C) and culture supernatants (S) from equal numbers of cells were immunoprecipitated with anti-κ antiserum. (**a**) Isolated proteins were reduced and resolved on a 15% SDS–PAGE. Positions of BiP and L chains (L) are indicated. Quantitation of the fluorograph was performed by scanning densitometry. (**b**) The amounts of L chains recovered from cell lysate (■) and culture supernatant (□) are expressed as percentage of the total amount of L chains isolated directly after the pulse. (**c**) The amount of coprecipitated BiP is expressed as percentage of BiP immunoisolated from an aliquot of the same lysate (compare Figure 2).

As expected, BiP was coprecipitated with intracellular L chains of NS1 cells (Figure 1a). It is noteworthy that the amount of coprecipitated radiolabelled BiP did not remain constant. Compared with the values obtained directly after the pulse, about twice as much labelled BiP was coprecipitated with L chains after 4 h of chase (Figure 1c). The slow increase in labelled coprecipitated BiP may reflect the accumulation of labelled BiP complexed to labelled and unlabelled L chains. Initially, the complex may be mainly composed of labelled L chains and pre-existing unlabelled BiP whereas later in the chase, newly synthesized unlabelled L chains associate with pre-existing labelled BiP. After 4 h of chase, ~6% of total labelled BiP was coprecipitated with L chains. However, the maximal amount of labelled BiP participating in the interaction with L chains was not yet reached at this time point. Since the specific activity of the BiP pool remained rather constant throughout the chase period (see below), this result may indicate that newly synthesized BiP has to undergo some post-translational modification in order to become a functional protein. We are currently testing this possibility.

When anti-BiP antibodies were used to perform immunoprecipitations from the same lysates, labelled L chains were co-isolated with labelled BiP (Figure 2a). Whereas the amount of isolated labelled BiP remained constant within the 4 h of chase, a time dependent decrease in the signal intensity of the L chain band was observed. About 13% of the total L chains were coprecipitated with BiP directly after the pulse. After 4 h of chase, 0.6% of the labelled L chains were co-isolated with the anti-BiP antibody.

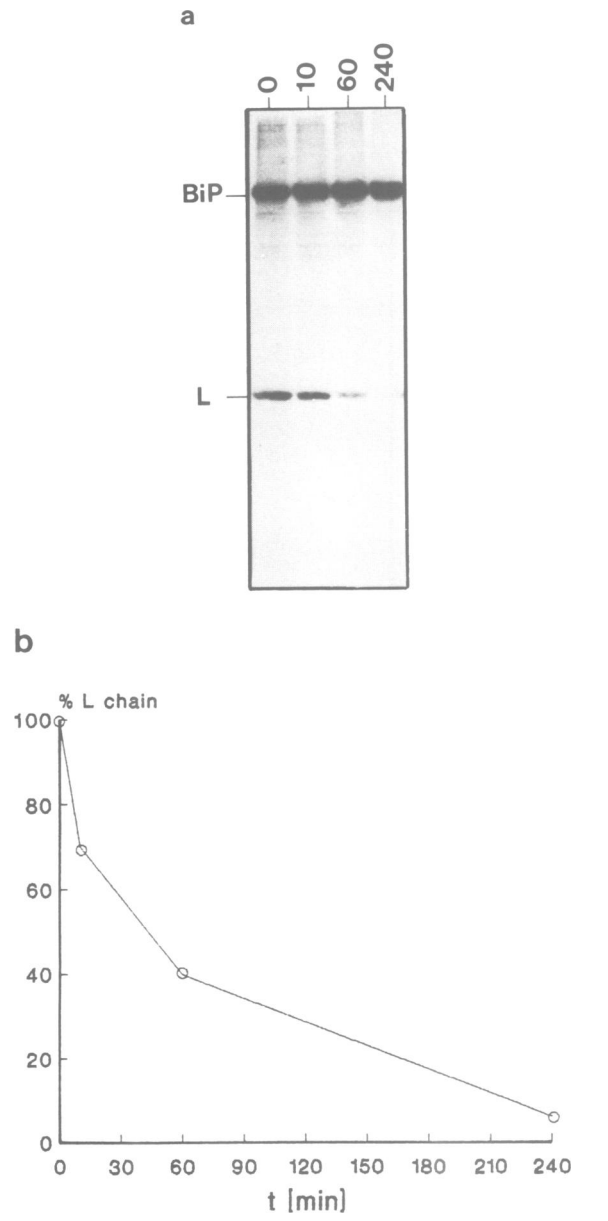

Fig. 2. Kinetics of BiP−L chain dissociation in non-secreting cells. Immunoprecipitation with anti-BiP antibodies was performed on aliquots of the same lysates as these used in the experiment described in Figure 1. (**a**) Isolated material was reduced, resolved on 15% SDS−PAGE and visualized by fluorography. Positions of BiP and L chains are indicated. (**b**) The amount of L chains coprecipitated at each time point is expressed as a percentage of the amount of L chains coprecipitated directly after the pulse.

From the microdensitometric scanning data, the biological half life of the BiP−L chain complex in NS1 cells was determined to be ~50 min (Figure 2b).

Since the half life of the L chains corresponded exactly to the half life of the BiP−L chain complex, it was important to know whether L chains were degraded in association with BiP. Therefore, we determined the biological half life of BiP in NS1 cells and compared it with that of BiP in a different cell line, X63Ag8.653. It is easily seen that the half life of BiP was not below 48 h in either of the cell lines (Figure 3). Thus, the turnover rate of BiP is rather slow which implies that the specific activity of the protein remains

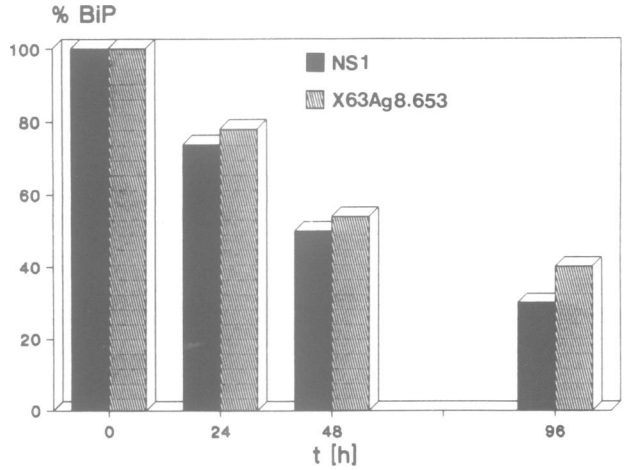

Fig. 3. Kinetics of BiP degradation in NS1 and X63Ag8.653 cells. NS1 (black bars) or X63Ag8.653 (stippled bars) cells were pulse labelled for 90 min with [^{35}S]methionine and chased for the times indicated. At each of the time points, identical volumes of the cultures were taken to prepare cell lysates which were either directly analysed on an SDS−PAGE or used for immunoisolation of BiP. Quantification of the signal intensities was performed by scanning densitometry of the fluorographs. The amount of labelled BiP detected at each time point is expressed as a percentage of the amount of labelled BiP isolated directly after the pulse. Note that the weakest signals (96 h) are those for which the error in determination is greatest.

nearly unchanged over a long period of time. Knowing that 3−6% of total BiP is involved in the interaction with L chains, half of the labelled BiP molecules should have disappeared after 13−26 h in case the protein was irreversibly lost after degradation of the complex. From this calculation it is clear that the disappearance of the BiP−ligand complex is not a rate limiting step in BiP degradation. This is confirmed by the finding that the biological half life of BiP was about the same in X63Ag8.653 cells which is an Ig chain loss parent cell line of NS1. In conclusion, these results demonstrate that L chains dissociate from BiP directly before they are degraded.

'Transient' interaction of BiP with L chains in the secreting J558L cells

In order to characterize the interaction of BiP with a secreted L chain, we analysed J558L myeloma cells which synthesize and secrete high levels of λ chains in the absence of H chains. Cells were pulsed for 30 min and chased for different times after which cell lysates or equivalent amounts of cell culture supernatant were analysed for the amount of labelled L chains. As in NS1 cells, the amount of newly synthesized L chains diminished continuously during the chase period (Figure 4a, left panel). However, the decrease in the intracellular level was compensated for by the amount of radiolabelled L chains recovered from the respective cell culture supernatant (Figure 4a, right panel). Indeed, L chains were secreted in a quantitative fashion (Figure 4b). More importantly, BiP was coprecipitated with the intracellular L chains in J558L cells (Figure 4a). In contrast to the results obtained from the analysis of NS1 cells, constant amounts of labelled BiP (~13% of the total) was co-isolated with L chains between 1 and 4 h of chase (Figure 4c). This indicated that steady state conditions were quickly established with respect to BiP−L chain complexes and that pre-existing

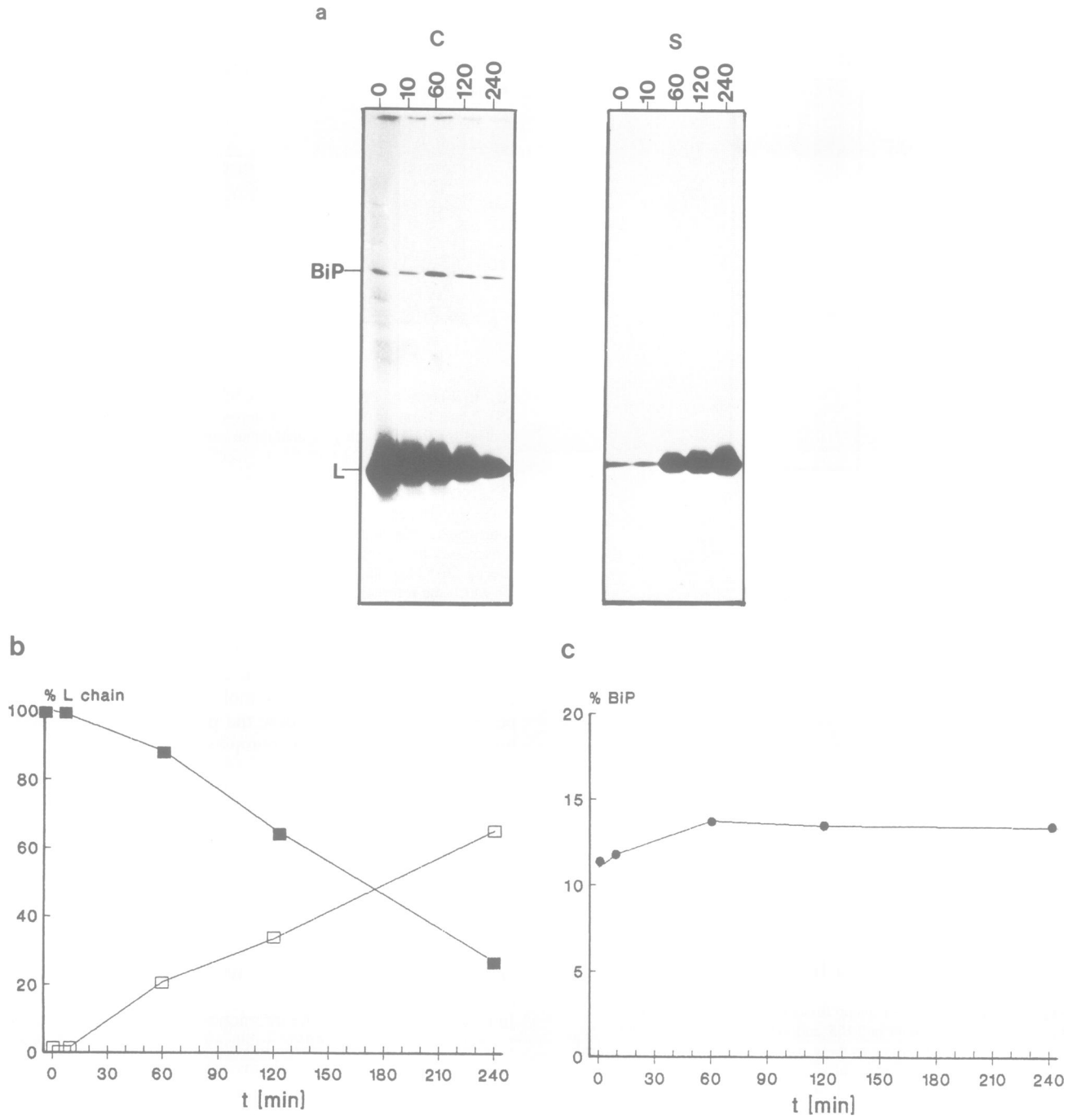

Fig. 4. Pulse–chase analysis of the interaction of BiP with L chains in J558L cells. Pulse labelling was performed as described in Figure 1. Anti-λ precipitated material was reduced and analysed on a 12.5% SDS–PAGE followed by fluorography. (**a**) Positions of BiP and L chains isolated from lysates (C) or cell culture supernatant (S) are indicated. Scanning data were obtained as described in Figure 1. (**b**) Shows the amounts of intracellular (■) and secreted (□) L chains, (**c**) the amount of coprecipitated BiP which is expressed as a percentage of BiP immunoisolated from an aliquot of the same lysate (anti-BiP precipitations are not shown).

BiP transiently interacted with newly synthesized L chains throughout the complete chase period.

In order to determine the half life of this complex, cells were pulse labelled for only 5 min and immunoprecipitations performed at earlier time points of chase. When immunoisolated L chains were analysed under non-reducing conditions, both a covalently linked dimeric and a monomeric form were detected (Figure 5a). Most of the dimers had already formed during the pulse since the ratio of the signal intensities did not change during the chase. As in the previous experiment, the amount of coprecipitated labelled BiP remained constant meaning that the steady state levels were already established at these time points. As expected, newly synthesized L chains interacted with BiP for only a very short period. When BiP was immunoisolated, labelled L chains were coprecipitated only at early time points of chase (Figure 5b). In fact, the half life of BiP–L chain complex in J558L cells was ~2 min (Figure 5c). Note that only L chain monomers were coprecipitated with BiP.

These results demonstrate the interaction of BiP with

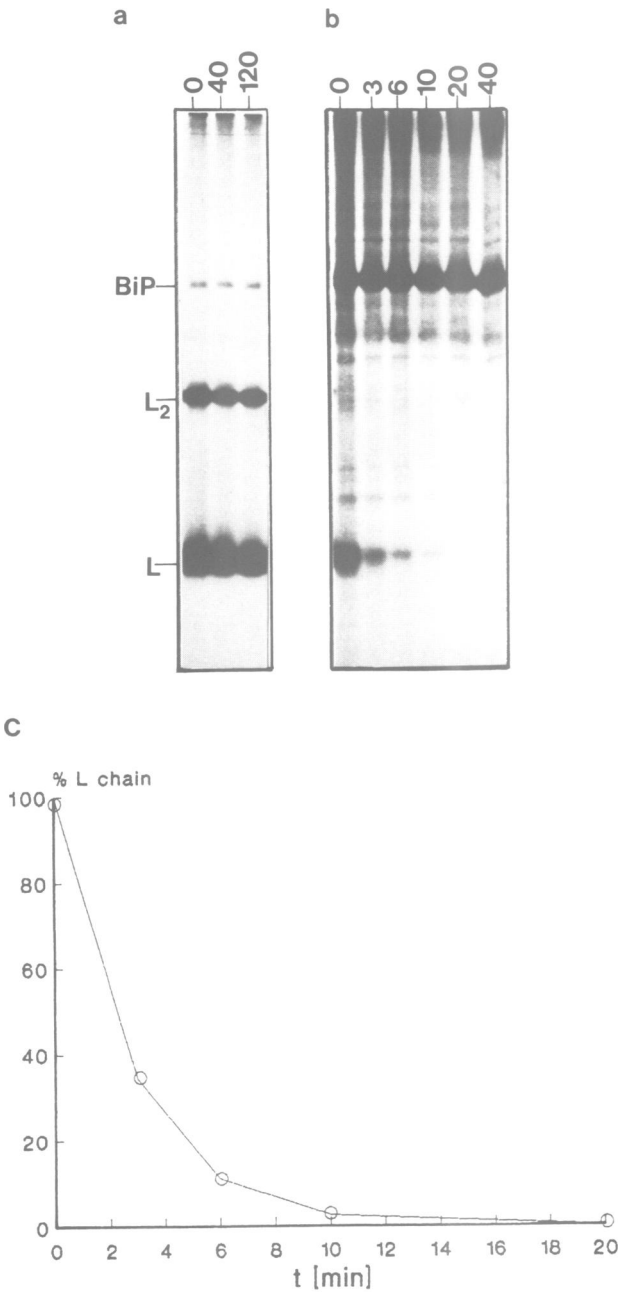

Fig. 5. Kinetics of BiP–L chain dissociation in secreting cells. J558L cells were pulsed for 5 min and chased for 0, 3, 6, 10, 20, 40 and 120 min as indicated by lane labels. Lysates were immunoprecipitated with (**a**) anti-λ antiserum or (**b**) anti-BiP monoclonal antibody. SDS–PAGE on 12.5% gels were performed under non-reducing conditions. Note that the fluorograph shown in (b) was exposed twice as long as the one in (a). The positions of BiP and L chains are indicated. (**c**) The amount of L chains coprecipitated at each time point is expressed as the amount of L chains coprecipitated directly after the pulse.

normal L chains which are subsequently secreted from the cells. As in NS1 and X63Ag8.653 cells, the half life of BiP was >48 h in J558L cells as well (data not shown). Knowing that 10–13% of total BiP is involved in the interaction with L chains, and that the complex has a half life of 2 min, the pool of labelled BiP would be exhausted at the latest after 40 min of chase if BiP only interacted once with L chains. However, the amount of radiolabelled BiP which coprecipitated with L chains after 4 h of chase did not

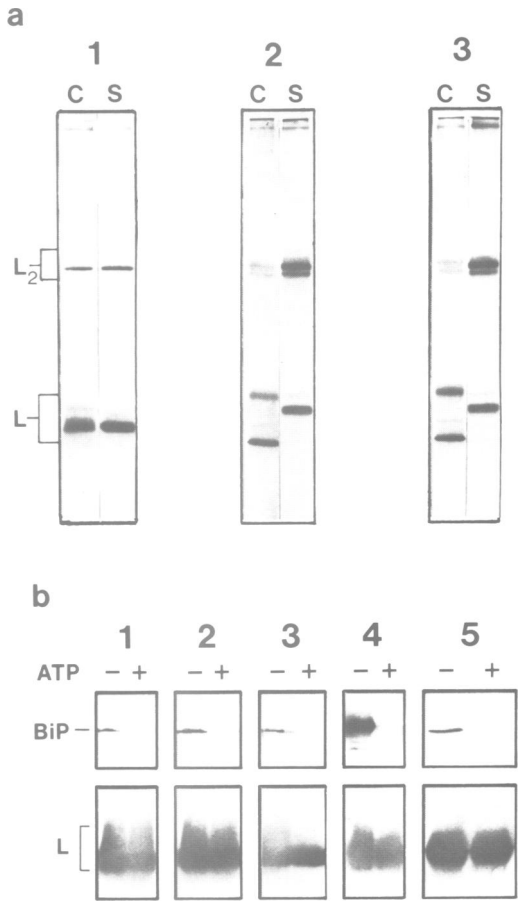

Fig. 6. Interaction of BiP with L chains is seen in several L chain secreting cells. (**a**) L chains from lysates (C) and culture supernatants (S) of 4F5.1.4 (1), Sp2.7 (2) and NORA4.8.40 (3) cells were isolated by immunoprecipitation and analysed in non-reducing Western blots; the electrophoretic mobilities of L chain monomers (L) and covalently linked dimers (L_2) are indicated. (**b**) Lysates of 4F5.1.4 (1), Sp2.7 (2), NORA4.8.40 (3), NS1 (4) and J558L (5) were treated (+) or not (–) with 5 mM MgATP prior to immunoprecipitation with anti-κ (1–4) or anti-λ antiserum (5). Reducing Western blots were developed with anti-L chain antisera (L) or anti-BiP antibodies (BiP). Note that all κ-producing cell lines were analysed on a single blot.

decrease compared with the value obtained directly after the pulse. Therefore, our results also prove that once dissociated from L chains BiP is able sequentially to bind newly synthesized L chains.

Interaction of BiP with L chains is a general phenomenon

In order to exclude the possibility that the interaction of BiP with secretable L chains was a peculiarity of the J558L myeloma, we additionally analysed other L chain secreting lines. Figure 6a demonstrates that all of the cell lines included in this study indeed secreted L chains. Since the Western blot analyses were performed under non-reducing conditions, different species of L chains were detected. Covalently linked dimers were usually found in lysates as well as in cell culture supernatants. In some cases however, this form was preferentially secreted (Sp2.7 and NORA 4.8.40 in Figure 6a). The different migration pattern of L chain monomers recovered from lysates or supernatants of Sp2.7 and NORA4.8.40 cells is due to the isolation procedure: when cells were lysed in the presence of culture medium, both

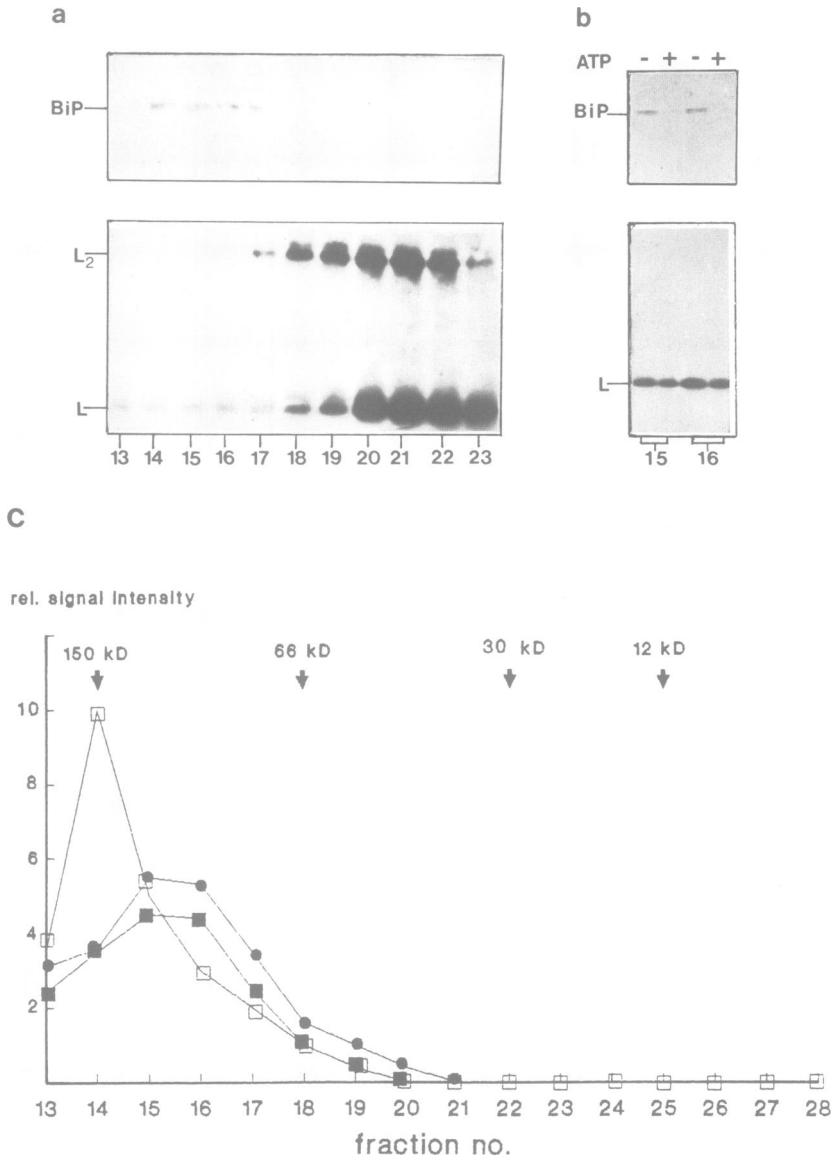

Fig. 7. Size fractionation of BiP and L chains of J558L cells. Total cellular proteins were size fractionated by gel filtration on Sephacryl S200. (**a**) Individual fractions were treated with anti-λ antiserum and immunoprecipitated material was analysed on Western blots for the presence of L chains (L) or BiP (BiP) under non-reducing conditions. (**b**) Fractions 15 and 16 were treated (+) or not (−) with 5 mM MgATP prior to immunoprecipitation and analysed on a Western blot under reducing conditions. (**c**) Additional experiments performed under conditions described in (a) were done to analyse the size distribution of BiP in untreated (□) or L chain enriched fractions from J558L (■) or NS1 (●) cells. The results are expressed in arbitrary units from microdensitometric scanning of Western blots. The markers used to calibrate the column were alcohol dehydrogenase (150 kDa), bovine serum albumin (66 kDa), carbonic anhydrase (30 kDa), and cytochrome *c* (12 kDa).

intracellular and secreted monomers migrated with identical electrophoretic mobility. In addition, only a single L chain signal was detected when the Western blot was performed under reducing conditions (data not shown). In this study, we did not analyse whether any of the monomers also derived from non-covalently associated dimers.

It is shown that BiP−ligand interactions are specifically dissociated *in vitro* by the addition of ATP (Munro and Pelham, 1986). In order to test whether this was also true for BiP−L chain complexes, the lysates derived from the different cells were treated with ATP prior to L chain isolation. The isolated material was analysed for the presence of L chains and BiP by Western blotting of an SDS−PAGE performed under reducing conditions. From Figure 6b, it is clearly seen that BiP is coprecipitated with all of the L chains in an ATP dependent fashion. The same number of κ producing cells were subjected to immunoprecipitation and subsequently analysed on a single blot. This allowed us to compare the relative signal intensities corresponding to the respective amounts of BiP coprecipitated with the various L chains. It is easily seen that, in NS1 cells, at least 10 times more BiP is associated with L chains presumably reflecting the accumulation of long lived BiP−L chain complex in these cells.

From these results we conclude that BiP specifically interacts with L chains and that this is an event common to all L chain producing cells. However, non-secreting cells exhibit much higher steady state levels of BiP−L chain complex when compared with L chain secreting cells. Note that the non-secreting line contains a higher steady state level

of BiP (Nakaki *et al.*, 1990; our unpublished results), so that the 6% of total BiP complexed with L chains in NS1 is—in absolute terms—more than the 13% bound to L chains in J558L cells (see Figures 1 and 5, respectively).

Characterization of a BiP–L chain complex

We have shown that BiP is associated with L chains in a transient fashion in cells which secrete L chains. In order to determine the molecular composition of the complex, we performed gel filtration analyses on a lysate of J558L cells and analysed the fractions for the presence of BiP and L chains in a Western blot performed under non-reducing conditions.

As can be seen from the lower part of Figure 7a, covalently linked L chain dimers were recovered from the gel filtration column in fractions 17–23 with a peak corresponding to a molecular size of ~40 kDa (for the size, compare Figure 7c). In these fractions, some L chains migrate as monomers indicating the existence of non-covalently associated dimers. However, L chain monomers were also present in fractions that corresponded to a higher molecular weight (Figure 7a, fractions 13–16). In these fractions, BiP was also stained (Figure 7a, upper part). The recovery of BiP was due to coprecipitation with L chains since the presence of ATP abolished the coprecipitation of BiP with L chains in the respective fractions (Figure 7b). When the lysate fractions were analysed without prior enrichment for L chains, the main portion of BiP was found in fraction 14 corresponding to a molecular weight of ~150 kDa (Figure 7c). Thus, the majority of BiP molecules are not in a monomeric form. Since L chain-associated BiP was eluted later from the column, we concluded that L chains are associated with BiP monomers. However, it is not clear whether one or two molecules of L chain participate in complex formation. We repeated the analysis using NS1 cells which are known not to contain L chain dimers (Ma *et al.*, 1990). In this experiment, BiP–L chain complex was recovered in the same fractions with a peak corresponding to a molecular weight of ~110 kDa (Figure 7c). An L chain containing band corresponding to this molecular size also appeared in an ATP dependent manner after SDS–PAGE analysis of chemically crosslinked lysate proteins (data not shown). We therefore conclude that the major portion of a BiP–L chain complex is composed of a BiP monomer bound to one molecule of L chain. However, our data do not exclude that a subfraction of the complex may contain further components which are not revealed in these analyses. Likewise, the experimental conditions used could lead to the release of additional components present in the physiological complex. We are currently investigating these points further.

Discussion

The physiological meaning of BiP–ligand interaction is not yet understood. It has been suggested that BiP acts as a part of a control system in the ER (Hurtley and Helenius, 1989). However, from an analysis of the fate of mutant VSV-G proteins it would seem that the function of BiP is not solely to retain misfolded proteins (Machamer *et al.*, 1990). On the other hand, it is clear that polypeptide chains have to be degraded in the case of permanent BiP ligands, e.g. L chains expressed in NS1 cells. In this case, we found a precise coincidence of BiP dissociation and degradation of the L chains which strongly indicates that Ig L chains are degraded in the ER. This is in line with the data described by Klausner and Sitia (1990) who have proposed a novel ER degradation pathway. However, BiP does not irreversibly signal the bound ligand to degradation since it was shown that BiP bound H chains can dissociate from BiP *in vivo* and be secreted as part of complete antibody molecules (Hendershot, 1990). In antibody producing cells, Ig H chains transiently interact with BiP and may indeed follow this assembly pathway (Bole *et al.*, 1986).

In fact, an increasing amount of evidence supports the view that BiP interacts with incompletely folded precursors which are released from BiP if the ligand achieves proper folding. Consistent with this view are the kinetics of BiP interaction and trimerization of VSV-G protein. At least 20% of newly synthesized VSV-G polypeptides bind to BiP but are only released from it when the protein is allowed to form correct intrachain disulfide bonds (Machamer *et al.*, 1990). BiP associates with newly synthesized incompletely folded states of the α-subunit of mouse muscle nicotinic receptor (Blount and Merlie, 1991). Transient association of BiP with immature viral hemagglutinin-neuraminidase (HN) may be part of the normal HN maturation pathway (Ng *et al.*, 1989).

Immunoglobulin L chains now represent a second example of BiP ligands which may exhibit permanent binding in case the appropriate assembly partner is not co-expressed. Thus, it is conceivable that a permanent type of BiP interaction always reflects a situation where the ligand is unable to undergo modifications which normally occur while the ligand is bound to BiP. This assumption is in line with the findings of Machamer and coworkers who showed that mutant VSV-G polypeptides which are unable to undergo correct glycosylation or disulfide bond formation remain stably bound to BiP. In contrast, transient interaction with BiP was observed for such folding mutants which were affected in later stages of domain formation (Machamer *et al.*, 1990).

Why are L chains of NS1 cells unable to dissociate from BiP and be secreted? Incorrect glycosylation as a possible reason for permanent BiP association does not apply for murine κ L chains. Obviously, the formation of covalently linked dimers is also not an absolute requirement for L chain secretion although this form is sometimes preferentially exported. Furthermore, the constant domain of κ is used in other L chains perfectly able to be secreted in the absence of H chains (see Sp2.7 and NORA4.8.40 in Figure 6). Therefore, the inability to be secreted must be caused by properties intrinsic to the variable (V) region of the NS1 L chain. However, the same V domain is able to pair with the V domains of different H chains giving rise to secreted antibody molecules (Köhler *et al.*, 1978). Apparently, secretion can occur whenever pairing of the L chains is possible. It is therefore very likely that L chains of NS1 cells are incapable of pairing with each other and, more importantly, that pairing is a prerequisite to the dissociation of L chains from BiP. Pairing of V domains is of essential importance in the formation of functional antibody molecules. Thus, the interaction of BiP with both Ig H and L chains probably ensures proper pairing and folding of these domains during the process of subunit assembly in the ER.

Our results show that a heterodimer is formed between BiP and newly synthesized L chain monomers. In the case of a transient interaction, BiP very rapidly dissociates from the L chain (2 min in J558L cells, Figure 5). Nevertheless, high steady state levels of BiP (at least 13%, Figure 5) are complexed with L chains. Assuming that each BiP molecule

has the potential to participate in this interaction (which is certainly not the case because BiP undoubtedly also interacts with other ligands) then total cellular BiP would be exhausted within a few minutes. We show that this is not the case. Thus, our results require that BiP is recycled after having released an L chain in J558L cells. Considering that BiP has a half life of >48 h, each molecule could theoretically interact on average >100 times with a newly synthesized L chain molecule in J558L cells before it is degraded. This catalytic way of acting is in line with the postulated chaperone function of BiP (Pelham, 1986; Ellis, 1987) and may also provide a reasonable explanation for its long biological half life. More importantly, these findings prove that BiP itself is not irreversibly modified during the process.

The physical stability of the BiP–L chain complex depends on salt and detergent concentrations in the buffers used for cell lysis and immunoprecipitation. A 2-fold increase in the amount of complex recovered from the cells was observed when detergent-free wash buffer with low salt concentration was used (M.R.Knittler and I.G.Haas, unpublished results). This indicates that our experimental values underestimate the real amount of BiP–L chain complex present in the respective cells. These findings also illustrate why experimental conditions sufficient to describe permanent BiP–ligand interactions may not always be appropriate to detect low steady state levels of BiP–ligand complexes when transient binding is concerned. The dissociation of the BiP–L chain complex in the presence of salt and detergent argues for a low affinity interaction between the two proteins. The same was also found for NS1 L chains which *in vivo* show a permanent and thus stable type of BiP interaction. From this, it is clear that the physical stability of the complex does not dictate the biological fate of the ligand.

The fact that the same BiP–ligand complex may exhibit different biological stabilities raises the question of which physiological circumstances are responsible for the induction of the respective dissociation. As discussed above, it may be the ability of the particular ligand either to assemble with another polypeptide chain or to fold properly on its own which causes its dissociation from BiP. However, since BiP is not directly involved in ligand degradation, permanent ligands also require a signal to be released from BiP. A hypothetical second component may attach to the ligand, induce its dissociation from BiP, and signal the polypeptide to the degradation pathway.

Both mechanisms of BiP–ligand dissociation are not mutually exclusive but may operate at different time points after formation of the complex. Ligands which form a long-lived complex with BiP are destined for degradation, whereas ligands which interact with BiP only for a very short time are not. This could explain why only few if any L chains follow the degradation pathway in J558L cells whereas all of the L chains are degraded in NS1 cells. Along this line, one could predict that ligands which interact with BiP for an intermediate period of time would be partly degraded and partly exported from the ER. Whatever the mechanism, potential BiP ligands could escape degradation by correctly folding in time.

A less static view of the two different types of BiP–ligand interaction would see the permanent type of binding as the result of an accumulation of many transient interactions, and all ligands would dissociate from BiP within the same time frame. Those ligands which do not find an appropriate partner chain would immediately reassociate with BiP, whereas ligands which locate a partner will be removed from the reaction.

Materials and methods

Cell culture

J558L is a H chain loss variant of the mouse plasmacytoma J558 (α,λ_1) (Oi *et al.*, 1983). NS1 is a plasmacytoma, which synthesizes but does not secrete κ chains (Köhler *et al.*, 1976). X63Ag8.653 is a parent of NS1 and does not express immunoglobulin chains (Kearney *et al.*, 1979). 4F5.1.4 (gift of D.Tarlinton) is an H chain loss variant of the hybridoma 4F5S (μ, κ) (Förster *et al.*, 1988). Sp2.7 is an H chain loss variant of the hybridoma Sp2 (γ_{2b}, κ) (Köhler and Milstein, 1976). NORA4.8.40 is an H chain loss variant of the hybridoma NORA4.8 (γ_{2b}, κ) (Haas and Wabl, 1984). All cells were maintained in RPMI 1640 supplemented with 10% FCS, 1000 U/ml penicillin and 1 mg/ml streptomycin.

Biosynthetic labelling and immunoprecipitation

Cells (4×10^6/ml) were starved for 2 h in methionine-free DMEM medium containing 10% dialysed FCS before [^{35}S]methionine was added for either 5 min (500 μCi/ml) or 30 min (300 μCi/ml). The chase was initiated by the addition of excess unlabelled methionine (2 mM). In long-term chase experiments, cells were diluted directly after the pulse and maintained under normal culture conditions. Aliquots were removed at various times of chase, cells were separated from supernatant and washed twice in ice cold PBS prior to solubilization in NET buffer (Kessler, 1975). Immunoprecipitations were performed from equivalent amounts of cell lysates or supernatants by adding an equal volume of immunoabsorbant buffer (200 mM H_3BO_3, 50 mM $Na_2B_4O_7$, 150 mM NaCl, 1% NP-40 and 0.1% ovalbumin; pH 8.3) and using anti-BiP antibodies (gift of L.Hendershot) or affinity purified goat antibodies to mouse Ig κ or λ L chains (SBA, Birmingham AL, USA) in combination with Protein A Sepharose. Precipitates were washed six times with borate–NaCl buffer (0.5% NP-40, 1 M NaCl, 25 mM $Na_2B_4O_7$, 0.1 M H_3BO_3, pH 8.3) and twice with 40 mM HEPES, pH 8.0. Samples were run on SDS–PAGE (Takacs, 1979) under reducing or non-reducing conditions. Gels were stained with Coomassie Brilliant Blue to control for precipitating antibodies. Fluorographs were obtained after different exposure times and the X-ray films scanned by microdensitometry using a Joyce–Loebl Chromoscan II. All autoradiographs show the whole gel. In the long-term pulse–chase experiments, quantification of immunoisolated material was corrected for exponential decrease of radiolabelled total cellular proteins determined at each time point of chase.

Size fractionation of cellular proteins and Western blot analysis

Cells were washed in PBS and lysed (10^7/ml) in NET buffer. Size fractionation of total cellular proteins (0.5 ml of lysate) was performed by gel filtration on Sephacryl S 200 (Pharmacia/LKB, Uppsala, Sweden). The fractions eluted from the column (1 ml) were either analysed directly or mixed with an equal volume of immune absorbent buffer prior to immunoprecipitation. Proteins were separated on SDS–PAGE and transferred onto nitrocellulose membranes using a Fastblot B 33 chamber (Biometra, Germany). Before blocking (PBS/0.5% dry milk powder/0.05% Tween 20), the membranes were occasionally divided into two parts (between 46 and 69 kDa). The upper part was treated with anti-BiP antibodies and a second biotinylated antibody, the lower part with biotinylated anti-mouse L chain antibodies; all antibodies were diluted in blocking buffer. Proteins were visualized after incubating the membranes with streptavidin–alkaline phosphatase using the bromochloroindolyl phosphate/nitro-blue tetrazolium substrate.

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