Mechanisms Targeting Apolipoprotein B100 to Proteasomal Degradation

Evidence That Degradation Is Initiated by BiP Binding at the N Terminus and the Formation of a p97 Complex at the C Terminus

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Objectives—In lipid-poor states, the ubiquitin-proteasomal pathway rapidly degrades misfolded apolipoprotein B100 (apoB) cotranslationally, although the mechanism of delivery from the ER to cytosolic proteasomes is poorly understood. Here we demonstrate key roles of BiP, an ER luminal chaperone, and p97, a cytosolic ATPase anchored to the ER membrane, in the targeting of apoB for proteasomal degradation.

Methods and Results—Using coimmunoprecipitations, we observed associations of apoB with BiP, p97, Derlin-1, VIMP, and the E3 ubiquitin ligase Hrd1 in HepG2 cells. BiP and p97 were found to bind apoB cotranslationally. Expression of C-terminal truncated apoB molecules in COS-7 cells showed an N-terminal region outside apoB15 and a C-terminal region found in apoB72 were required for BiP and p97 binding, respectively. Interestingly, overexpression of dominant negative p97 demonstrated that the ATPase activity of p97 was essential for proteasomal degradation of apoB but not for apoB binding. However, p97 activity did not appear to affect the N terminus of apoB, which may be cleaved before degradation.

Conclusions—These data suggest that p97 and BiP play critical roles in the cotranslational delivery of apoB to proteasomes and formation of a degradative complex. Proteasomal degradation appears to selectively target apoB molecules with large C-terminal domains. **(*Arterioscler Thromb Vasc Biol*. 2009;29:579-585.)**

Key Words: apolipoprotein B ■ degradation ■ p97 ■ BiP ■ proteasome

Apolipoprotein B100 (apoB) is a 550-kDa protein synthesized by hepatocytes whose production is regulated mainly by cotranslational degradation via the ubiquitin-proteasomal pathway.[1] During translation, the N terminus of apoB is translocated into the ER lumen[2] where BiP, a chaperone protein, stabilizes hydrophobic sites.[3,4] In the presence of sufficient microsomal triglyceride transfer protein (MTP) activity and lipid species, apoB is lipidated and may be fully translated, properly folded, glycosylated, and eventually secreted as a very low density lipoprotein (VLDL).[5,6] In the absence of proper lipidation, BiP is believed to bind apoB tightly and help target it for proteasomal degradation from within the ER.[7] The mechanism is not clear, but BiP overexpression or upregulation appear to induce ubiquitination and degradation of apoB.[7] Because proteasomes are located in the cytosol or associated with the cytosolic face of the ER, a process is required to deliver apoB to proteasomes.

There has been controversy concerning whether proteasomal degradation of apoB requires full translocation of apoB into the ER lumen followed by complete retrotranslocation of luminal apoB.[8,9] It is unclear how such a large protein, which is potentially partially lipidated and glycosylated, could cross the ER membrane into the cytosol. However, there is also evidence of prolonged association of apoB with the ribosome and translocon and impaired translation and translocation of apoB in the absence of MTP activity or sufficient lipids,[10] suggesting apoB would not undergo complete translocation and then complete extraction. Strong evidence also indicates there is very little cytosolic exposure of the N terminus of apoB[11] whereas the majority of the apoB molecule is exposed to the cytosol during translation, especially in situations resulting in poor apoB lipidation.[12] One possibility is that under conditions that impair apoB lipidation and translocation, the N terminus is cleaved,[12–14] leaving the cytosolic portion to be degraded by the proteasome. Regardless of the size of apoB molecules that are degraded, the factors involved in the targeting to cytosolic proteasomes are unknown.

p97 (valosin-containing protein [VCP]) is a cytosolic member of the ATPases associated with various cellular

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activities (AAA) family. It has roles including membrane fusion, cell cycle regulation, stress response, programmed cell death, and B and T cell activation, depending on its associated cofactors.[15,16] p97 has also been shown to extract some proteasomal substrates from the ER lumen or ER membrane into the cytosol.[17–19] p97 is anchored to the ER membrane by interactions with transmembrane proteins such as Derlin-1, VCP interacting membrane protein (VIMP), and E3 ubiquitin ligases such as gp78 and Hrd1.[20] Derlin-1 has been shown to receive some substrates before they are passed onto p97, and VIMP partially mediates the interaction between Derlin-1 and p97.[20] The E3 ligase that polyubiquitinates apoB has been proposed to be gp78 based on an increase in apoB ubiquitination and degradation upon gp78 overexpression in HepG2 cells,[21] but the roles of other E3 ligases such as Hrd1 in apoB degradation have not been investigated.

In the present study, we provide evidence that cotranslational degradation of apoB may be initiated by strong binding of BiP to the N terminus, followed by the association of p97 with the C terminus and the involvement of a degradation complex consisting of p97, Derlin-1, VIMP, and Hrd1 in the targeting of apoB for cytosolic proteasomal degradation. Our data suggest the ER luminal N terminus of apoB may not interact with p97 or its transmembrane partners or undergo proteasomal degradation.

Methods

For more details, please see the supplemental materials (available online at http://atvb.ahajournals.org).

Cell Culture

HepG2 cells and COS-7 cells were obtained from the American Type Culture Collection and were maintained in DMEM supplemented with FBS and penicillin/streptomycin. COS-7 cells were transfected with apoB constructs with various C-terminal truncations (apoB15, apoB29, apoB48, apoB72)[22] using Lipofectamine (Invitrogen).

Transduction of HepG2 Cells With p97 Adenoviruses

HepG2 cells were transduced with adenoviruses encoding β-galactosidase (β-gal),[23] His-tagged wild-type p97, or His-tagged dominant negative p97 defective in ATP binding (K524A, referred to as KA) or ATP hydrolysis (E305Q and E578Q, referred to as QQ). p97 adenovirus expression was controlled by a tet-off system requiring coexpression of a tTA adenovirus.

Radiolabeling Experiments

HepG2 cells were starved of methionine and cysteine for 1 hour in the presence or absence of 25 μmol/L MG132 and labeled for 1 hour with 100 μCi/well ^{35}S-methionine. Cells were lysed, aliquots were used for p97 immunoblotting, and the remaining cell lysates were immunoprecipitated for apoB and albumin in series. Radiolabeled apoB and albumin were visualized by phosphorimaging of SDS-PAGE gels.

Trypsin Digestion of HepG2 Cells Transduced With p97 Adenoviruses

HepG2 cells were radiolabeled, permeabilized, and trypsin digested as described previously.[24] ApoB was immunoprecipitated, run on an SDS-PAGE gel, and fragments were visualized by phosphorimaging.

Crosslinking, Coimmunoprecipitation, and Immunoblotting

Cells were subjected to crosslinking with dithiobis (succinimidyl propionate, (DSP), and apoB was immunoprecipitated with antihuman apoB antibody. Immunoblotting was performed as described previously[23] using primary antibodies against apoB, albumin, ubiquitin, Derlin-1, VIMP, p97, BiP (α-KDEL), Hrd1, apoAI, His-tag, and β–actin.

Results

ApoB Associates With BiP, p97, Derlin-1, VIMP, and Hrd1

The interactions of BiP and p97 with apoB and the crosslinking/coimmunoprecipitation protocol were first examined by immunoprecipitating apoB from crosslinked or non-crosslinked HepG2 cells and immunoblotting for apoB, BiP, p97, and albumin. The efficiency of the apoB immunoprecipitation was lower with crosslinking, possibly because of steric hindrance of antibody binding to apoB when surrounded by its associated proteins (Figure 1A). However, crosslinking helped preserve the interactions of p97 and BiP with apoB, which were probably not maintained without crosslinking because of the solubilization conditions used (solubilizing buffer consisted of PBS containing 1% Nonidet P-40, 1% deoxycholate, 5 mmol/L EDTA, 1 mmol/L EGTA, 2 mmol/L phenylmethylsulphonyl fluoride and 0.1 mmol/L aprotinin). The fact that albumin, an abundant protein in the secretory pathway of HepG2 cells, was not crosslinked to apoB suggests that the observed interactions of BiP and p97 with apoB were true associations and not artifacts caused by excessive crosslinking.

Under these experimental conditions, several factors involved in proteasomal targeting including BiP, p97, Derlin-1, VIMP, and Hrd1 were found to coimmunoprecipitate with

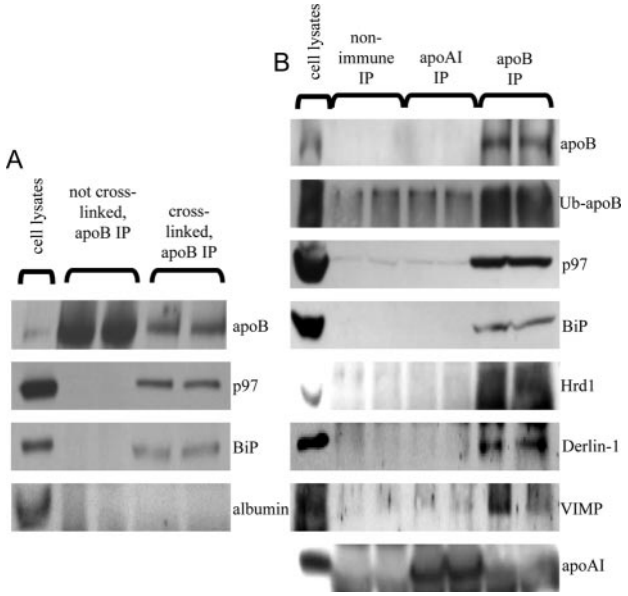

Figure 1. Identification of apoB binding partners. A, HepG2 cells were crosslinked or not crosslinked, apoB was immunoprecipitated, and immunoblotting was performed. B, HepG2 cells were crosslinked, immunoprecipitated using antibodies against apoB or apoAI or nonimmune antibodies, and immunoblotting was performed.

apoB (Figure 1B). Control immunoprecipitations using nonimmune goat serum or antibodies against apoAI demonstrated specificity in the associations of these factors with apoB. Ubiquitin immunoblotting indicated that some apoB was ubiquitinated, which is consistent with the involvement of these factors in the targeting of apoB molecules for proteasomal degradation.

BiP Binds an N-Terminal Region of ApoB, Whereas p97 Binds a C-Terminal Region of ApoB

To gain insight into the apoB regions associating with BiP and p97 and better understand the topology of apoB as it is targeted to the proteasome, apoB constructs with various C-terminal truncations were expressed in COS-7 cells, which are easy to transfect and do not express apoB endogenously. After allowing time for expression, associations of p97 and BiP with the truncated forms of apoB were examined. BiP associated with apoB29, apoB48, and apoB72, but not apoB15, whereas p97 associated only with apoB72 (Figure 2). Consistent with these results, apoB100 from HepG2 cells strongly associated with BiP and p97. A prominent apoB48-like protein was produced upon expression of apoB72 in COS-7 cells, which similarly to apoB48 consists of the N-terminal portion of apoB100.[22] Although it is possible that p97 was associating with the apoB48-like protein rather than apoB72, we do not believe this to be the case because p97 did not associate with apoB48 and the apoB48-like protein was similar to apoB48 in sequence, size, and amount expressed.

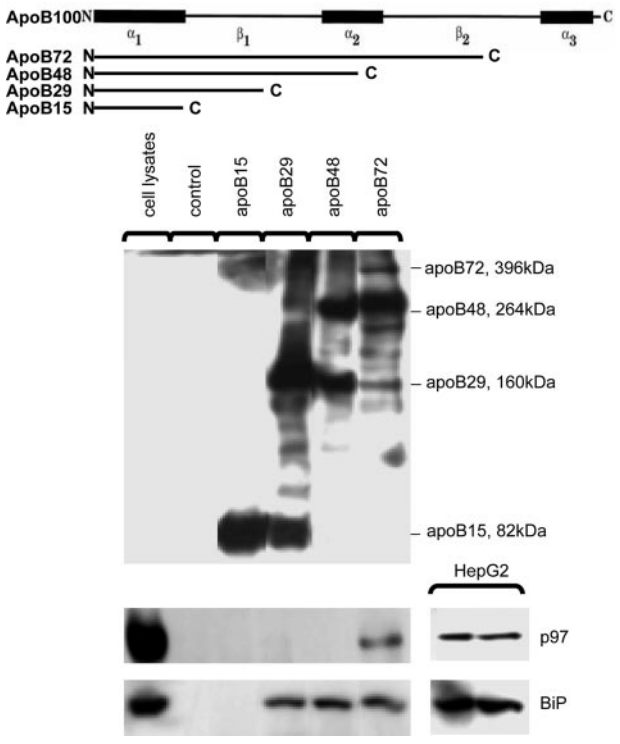

Figure 2. Determination of the apoB regions binding BiP and p97. COS-7 cells transfected with various apoB vectors, and nontransfected COS-7 or HepG2 cells were crosslinked and immunoprecipitated for apoB before immunoblotting for apoB, BiP, and p97 was performed.

BiP and p97 Sequentially Bind Newly Synthesized ApoB Cotranslationally

After identifying the regions of apoB that associate with BiP and p97, the next step was to further characterize the binding of these proteins to apoB by performing cycloheximide chase experiments to inhibit protein synthesis and follow the interactions over the course of the experiment. These experiments were performed to determine whether BiP and p97 were associating with a newly synthesized pool of apoB, which would disappear upon cycloheximide treatment, or a non-newly synthesized pool of apoB that might be subjected to some form of degradation or secreted. As shown in Figure 3A, simply blotting for total apoB mass without radiolabeling showed that apoB appeared to be quite stable regardless of cycloheximide or MG132 (proteasomal inhibitor) treatment, indicating that the visible bands might represent mainly posttranslational pools of apoB unlikely to associate with p97 or BiP. The nascent polypeptide chains susceptible to proteasomal degradation may have been present in quantities too low to be visible without radiolabeling. Once HepG2 cells were treated with cycloheximide for 1 hour, the associations of p97 and BiP with apoB disappeared, probably because these proteins bound unstable apoB molecules that were rapidly degraded or released into the cytosol (Figure 3A, left). In addition, blocking new protein synthesis with cycloheximide prevented formation of nascent apoB chains and thus any interaction with BiP or p97. MG132 treatment helped preserve the association of p97 with apoB, although not completely. Following 1 hour of cycloheximide treatment in the presence of MG132, only a slight association of p97 with

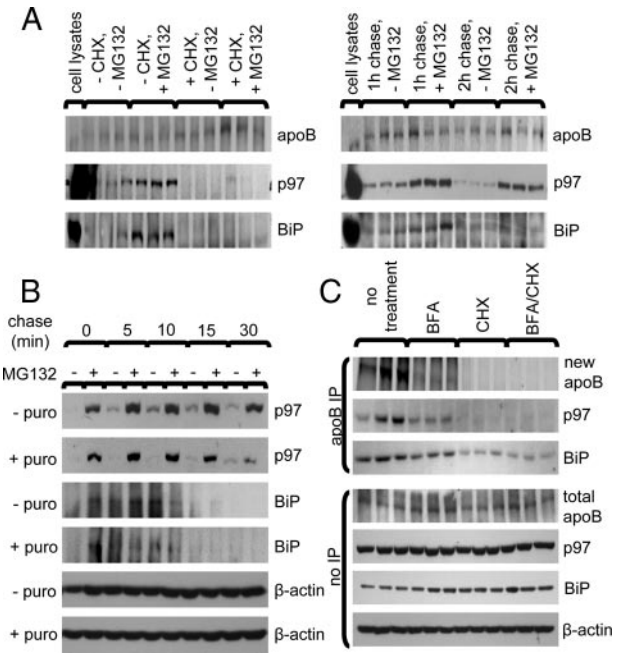

Figure 3. Characterization of apoB binding in HepG2 cells treated with cycloheximide and MG132 for 1 hour (A, left), washed, and incubated (A, right); MG132 for 30 minutes, then puromycin for 7.5 minutes, before being labeled with ^{35}S-methionine for 5 minutes and chased (B); or brefeldin A and cycloheximide for 1 hour and 45 minutes, respectively, before 1-hour incubation with ^{35}S-methionine (C). A, B, and C, Cells were crosslinked and immunoprecipitated for apoB.

apoB was observed, perhaps because of incomplete inhibition of proteasomal degradation by MG132. It is unclear how closely extraction from the ER membrane and proteasomal degradation are coupled and to what extent inhibition of degradation would interfere with the delivery of apoB to proteasomes by p97. MG132 had a less dramatic effect on the binding of BiP to apoB. The fact that the associations of p97 and BiP with apoB returned once cycloheximide was washed out and translation was reinitiated (Figure 3A, right) could indicate that these proteins bound a newly synthesized apoB pool, although such long chase times make this difficult to state conclusively.

To determine more conclusively whether p97 and BiP were binding newly synthesized apoB and to try to identify at what point after translation initiation the binding occurred, an experiment similar to that shown in Figure 3A was performed, but puromycin was used to inhibit translation, the proteins were radiolabeled, and shorter chase times were used. After puromycin treatment and wash-out, approximately 30 minutes were required for full-length radiolabeled apoB to appear (results not shown), and therefore apoB was in a cotranslational state before that point. Previously, apoB translation has been shown to take 15 to 20 minutes,[25] but after puromycin treatment, translation would initially be delayed while ribosomal subunits reassociated. Under these experimental conditions, in cells treated with puromycin, binding of BiP to apoB occurred early during the chase period (by approximately 5 minutes) and seemed to disappear toward the end of the chase period (Figure 3B). Even in the absence of puromycin, binding of BiP to apoB seemed to occur at early time points despite continuous apoB synthesis. In contrast, binding of p97 to apoB seemed to occur at chase times of approximately 15 to 30 minutes in the presence of puromycin. MG132 treatment preserved binding of p97 to apoB at early chase times even in the presence of puromycin, likely because MG132 treatment was initiated before puromycin treatment, maintaining interactions of apoB with p97 that existed before puromycin treatment. Without puromycin, p97 associated with apoB at all chase times. Overall, these results suggest that BiP and p97 bind newly synthesized apoB cotranslationally and that BiP may associate with apoB before p97.

To further confirm that BiP and p97 associate with apoB cotranslationally, nascent apoB molecules were eliminated from the cell by cycloheximide treatment, whereas other forms of apoB were maintained in the ER by inhibiting ER to Golgi transport with brefeldin A. Based on the apoB immunoblot, some apoB remained in the cell with cycloheximide and brefeldin A treatments, but it was not newly synthesized based on the lack of ^{35}S-methionine incorporation (Figure 3C). In addition, upon elimination of newly synthesized apoB by treating with cycloheximide, p97 could no longer be coimmunoprecipitated with apoB even in the presence of brefeldin A, confirming the cotranslational association of apoB with p97. Although the binding of BiP to apoB did not disappear completely on cycloheximide treatment, there was a considerable drop in the association, indicating that BiP binds apoB cotranslationally, but that the interaction may be maintained for a longer period of time than with p97. The fact that the expression of BiP and p97 did not decrease after cycloheximide treatment demonstrates that it was the lack of apoB synthesis that resulted in reduced coimmunoprecipitation of these proteins with apoB rather than a reduction in their levels.

p97 Does Not Appear to Act on the N Terminus of ApoB

Next, the role of p97 in extraction of apoB from the ER was investigated by performing trypsin protection experiments in permeabilized HepG2 cells transduced with no adenovirus, a β-gal adenovirus, or a KA or QQ dominant negative p97 adenovirus. KA (K524A) and QQ (E305Q, E578Q) are mutated forms of p97 defective in ATP binding and ATP hydrolysis, respectively. Using this approach, apoB domains within the ER lumen would be protected from digestion whereas regions protruding from the ER would be cleaved by trypsin. These experiments were meant to give qualitative information regarding the size of the fragments produced under each adenovirus condition rather than quantitative results concerning the amount of the fragments produced. The N-terminal apoB fragments protected within the ER from trypsin digestion did not appear to differ in size regardless of the adenovirus used to transduce the cells (comparing lanes 2, 5, 7, and 9 of Figure 4). It can also be seen that fragments approximately 70, 120, and 220 kDA in size were much more prominent after trypsin digestion (lanes 2, 5, 7, and 9 compared to lanes 1, 4, 6, and 8), similar to previous findings.[11] Even with longer chase times of up to 20 minutes, which increased the length of radiolabeled apoB molecules, the trypsin-resistant banding patterns were similar between cells expressing dominant negative p97 or β-gal as a control (results not shown). Corresponding results were obtained whether permeabilized cells or isolated microsomes were

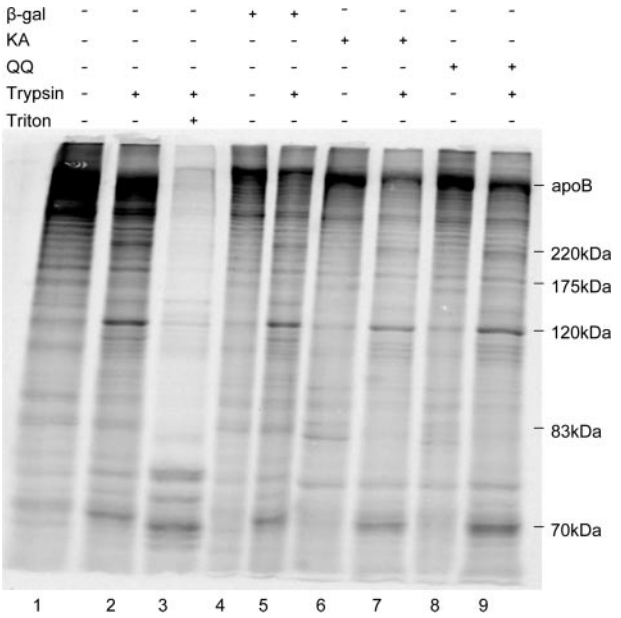

Figure 4. Cytosolic exposure of apoB. HepG2 cells transduced with adenoviruses were labeled with ^{35}S-methionine for 5 minutes and chased for 5 minutes before permeabilization and trypsin treatment. ApoB fragments were immunoprecipitated and visualized by phosphorimaging. Triton X-100 solubilized membranes, allowing trypsin digestion of all proteins.

treated with trypsin or proteinase K (unpublished data, 2008 Angela Rutledge). These findings suggest the N terminus of apoB was not affected by the presence or lack of p97 function. It is possible that the N terminus of apoB may be cleaved within the ER lumen from the remainder of the apoB molecule and that p97 may act on the portion remaining on the cytosolic face of the ER.

ATPase Activity of p97 Is Not Required for ApoB Binding, But Is Essential for Proteasomal Degradation of ApoB

We also investigated the importance of the ATPase activity of p97 in apoB binding. Adenoviruses encoding wild-type, ATP binding-defective, or ATP hydrolysis-defective forms of His-tagged p97, or β-gal were expressed in HepG2 cells, and some cells were treated with MG132. Complexes containing His-tagged p97 were pulled down with nickel-agarose columns. As shown in Figure 5A, exogenous His-tagged p97 was expressed well and pulled down well. Interestingly, apoB associated with mutant p97 as well as (or possibly better than) wild-type p97, indicating that binding of p97 to apoB did not require functional ATPase activity. ApoAI was not pulled down by the nickel-agarose columns, which demonstrates specificity in the pull-down of apoB with p97.

We examined the necessity of the ATPase activity of p97 in apoB degradation by radiolabeling HepG2 cells expressing β-gal or p97 defective in ATP binding or hydrolysis. Upon overexpression of dominant negative p97 (Figure 5B, left), a considerable accumulation of radiolabeled apoB relative to radiolabeled albumin was observed (Figure 5B, right). With MG132 treatment there were no statistically significant differences in the normalized apoB levels between the different adenovirus groups, suggesting proteasomal degradation was already inhibited by the dominant negative adenoviruses and that MG132 treatment only raised the apoB levels in the nontransduced and β-gal groups to the same level as the groups with mutated p97. The p97 adenoviruses were under the control of a tet-off system, and therefore doxycycline treatment turned off their expression, providing confirmation that their effects on apoB were attributable to overexpression of mutant p97 and not a nonspecific event. Wild-type p97 was also overexpressed in HepG2 cells, but did not appear to influence apoB accumulation (unpublished data, 2008 Angela Rutledge).

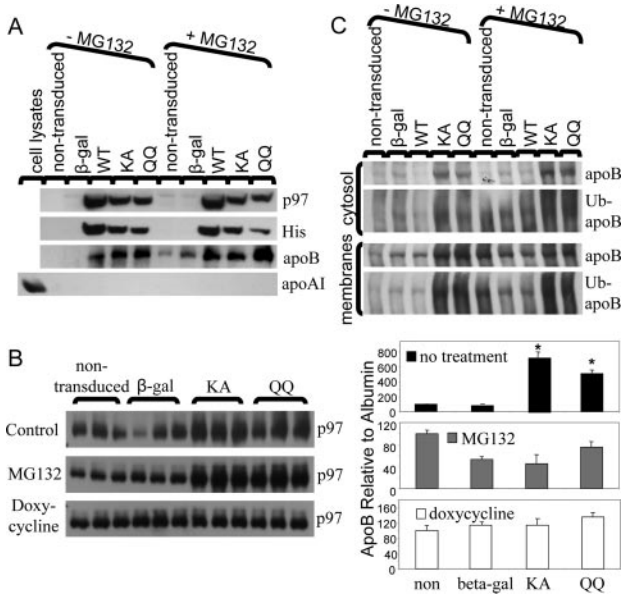

Figure 5. Role of ATPase activity. HepG2 cells transduced with adenoviruses were: treated with MG132 for 2.5 hours before crosslinking, His-tag pull-down, and immunoblotting (A); incubated with or without doxycycline for 2 days and treated with or without MG132 during a 1-hour pulse with ^{35}S-methionine (B, left, p97 expression; B, right, quantification of phosphorimaging bands); or treated with MG132 for 1 hour and permeabilized before immunoprecipitation of apoB from membrane and cytosol fractions (C). *Significantly different from controls ($P<0.05$).

Next, we investigated whether the apoB that accumulated on disruption of p97 function was ubiquitinated and where in the cell the apoB accumulated. Cytosol and membrane fractions were collected from digitonin-permeabilized HepG2 cells expressing the various p97 adenoviruses or controls that had been treated with MG132 for 1 hour or untreated. Figure 5C shows an increase in ubiquitinated apoB levels in cells expressing dominant negative p97 or in cells treated with MG132, suggesting that in both cases the apoB was ubiquitinated and ready to be degraded, but that the degradation process could not be completed. In addition, it appears that the majority of apoB that accumulated upon p97 inhibition was associated with the ER/membrane fraction rather than the cytosol, indicating that p97 activity is required for apoB (or at least the C-terminal portion of apoB) to be released from the ER membrane into the cytosol for degradation. Clearly, the ATPase activity of p97, although not needed for apoB binding or ubiquitination of apoB, was required for proteasomal degradation of apoB to prevent accumulation of ubiquitinated apoB at the ER.

Cellular ApoB Accumulated upon Inhibition of p97 Function Is Secretion-Incompetent and Induces ER Stress

Please see online supplemental results and supplemental Figure I.

Discussion

HepG2 cells have an overactive MEK/ERK signaling pathway that appears to be responsible for a defect in microsomal triglyceride availability.[26] As a result, HepG2 cells have impaired apoB lipidation, secrete mainly low density lipoproteins rather than VLDL,[26,27] and degrade most apoB molecules through the proteasomal pathway,[28] making them an excellent model for the study of proteasomal degradation of apoB.

Despite the myriad studies using HepG2 cells to study apoB degradation, many gaps remain in our knowledge of how apoB is delivered to cytosolic proteasomes. BiP has been demonstrated previously to play a role from within the ER in the targeting of terminally misfolded apoB for proteasomal degradation,[7] but the other factors involved have remained largely unknown. We were able to demonstrate associations of apoB with BiP, p97, Derlin-1, VIMP, and Hrd1. These results raise the possibility that Hrd1 could be an E3 ligase for

apoB, but of particular interest is the binding of p97 to apoB. It appears that both BiP and p97 associate with apoB undergoing cotranslational proteasomal degradation and that the interaction of BiP with apoB slightly precedes the binding of apoB to p97. This order is consistent with nascent apoB molecules being partially synthesized and inserted into the ER lumen, hydrophobic areas being stabilized by BiP, and if MTP cannot properly lipidate apoB, apoB being targeted to p97 and proteasomal degradation.

It was also observed that BiP associated with an N-terminal region of apoB not found in apoB15. An amphipathic C-sheet in the area between 19% to 20.1% of apoB100 has been proposed to initiate lipid binding to apoB.[22] It is possible that BiP binds this region or a hydrophobic region in the β1 domain (formed by the N-terminal 22% to 48% of apoB100).

p97 appeared to associate with a C-terminal region of apoB contained in apoB72 or larger molecules. Previously, compared to apoB72 or apoB100, apoB48 or smaller molecules were found to be relatively resistant to trypsin digestion of microsomes, indicating efficient translocation and little cytosolic exposure of the smaller apoB constructs.[29] When apoB is properly lipidated, translation and translocation rates may be such that the C terminus quickly enters the ER lumen and cannot associate with p97. However, under poor lipidation conditions, as apoB translocation is arrested and the translation rate exceeds the translocation rate, cytosolic exposure of apoB increases, possibly allowing p97 to access a C-terminal region of apoB.[8,10,22] Translocation arrested apoB has been found in a topology with its N terminus in the ER lumen, where BiP is located, and its C terminus in the cytosol, where p97 is found,[12] consistent with our observations that BiP and p97 were associating with N- and C-terminal regions of apoB, respectively.

We also gained insight into the function of p97 in apoB degradation. By overexpressing p97 defective in ATP binding or hydrolysis, we determined that apoB binding occurred independently of ATPase activity. However, ATPase activity of p97 was essential to prevent accumulation of ubiquitinated apoB at the ER by allowing its release into the cytosol and proteasomal degradation. Previously p97 has been found to bind nonubiquitinated substrates, but upon binding of p97 and its cofactors Ufd1 and Npl4 to polyubiquitin chains, a high affinity interaction occurs, activating the ATPase activity and fuelling extraction of substrates into the cytosol.[19] Also, polyubiquitination and cytosolic factors such as ATP have been shown to be important for proteasomal degradation of apoB, which could be attributable in part to the requirements of p97.[30] These findings are consistent with ATPase activity of p97 being necessary to deliver ubiquitinated apoB to proteasomes.

Because of the importance of p97 in apoB degradation and the outcomes of p97 inhibition, we expected overexpression of wild-type p97 in HepG2 cells to promote proteasomal degradation of apoB. However, there appeared to be no effect on apoB levels, likely because of the already high p97 expression ($\approx$1% of total cytosolic protein) and the high levels of proteasomal degradation of apoB in HepG2 cells.[28] p97 was unlikely to be a limiting factor, and even if there had been an increase in apoB degradation upon p97 overexpression, it would have been difficult to detect in this model.

Another clue about the mechanism of the action of p97 on apoB was gained by treating permeabilized HepG2 cells transduced with mutant p97 adenoviruses or controls with trypsin. Inhibition of p97 function did not seem to affect the size of apoB fragments protected in the ER lumen. Impairment of apoB lipidation appears to consistently arrest translocation at specific apoB domains. Previously, apoB translocation was found to be regulated by amphipathic β1 (22% to 48% of apoB100) and β2 (56% to 89% of apoB100) domains.[31,32] In experiments involving isolated microsomes or permeabilized cells either untreated or treated with proteinase K or trypsin, N-terminal fragments of apoB 69 to 70, 85, 120, and 220 kDa in size were found protected within the ER.[11–14] An endogenous N-acetylleucylleucylnorleucinal (ALLN)-sensitive protease may generate 69- to 70-kDa and 85-kDa fragments. The 69- to 70-kDa apoB fragment seems to be degraded within the ER by an ALLN-sensitive process, whereas the 85-kDa fragment undergoes degradation within the ER lumen by an ALLN-insensitive protease or secretion.[12–14] In addition, a prominent N-terminal apoB fragment approximately 80 kDa in size is secreted from COS-7, McArdle RH-7777, and HepG2 cells overexpressing ER-60, an ER-luminal chaperone protein/cysteine protease (unpublished data, 2008 Wei Qiu). It is possible that BiP may interact with the N-terminal fragment of apoB and that the association may be prolonged compared to the interaction of p97 with the remainder of the apoB molecule and its subsequent degradation. This could account for the maintenance of some of the association of BiP with apoB after elimination of newly synthesized apoB in Figure 3C. With so much evidence supporting regulated arrest of apoB translocation and cleavage of the N terminus under conditions of impaired apoB lipidation, it is understandable that p97 did not appear to affect translocation or degradation of the N terminus of apoB. p97 would likely be involved after cleavage of the N terminus in delivery of the cytosolic ubiquitinated portion of apoB to proteasomes for degradation.

Results presented here have provided insight into the mechanism by which lipid-deficient apoB comes to be degraded by proteasomes, but have left many details unknown. It is still not clear how BiP promotes proteasomal degradation of apoB, although it appears to associate with the N terminus of apoB, which may be cleaved cotranslationally, leaving a large cytosolic portion to be degraded. ER transmembrane proteins such as Derlin-1, VIMP, and Hrd1 help tether p97 to the ER,[20] forming a proteasomal targeting complex. Polyubiquitination of apoB by gp78 or Hrd1 would activate the ATPase activity of p97,[19] perhaps allowing p97 to pull apoB away from the translocon or other associated factors or otherwise make apoB more accessible to the proteasome. More studies are necessary to further characterize these and other components of the complex targeting apoB molecules to the proteasome.

Note Added in Proof

A very recent study by Roger McLeod and colleagues[33] has also reported an important role for p97 in proteasomal degradation of apoB. Knock-down of p97 in HepG2 cells appeared to result in increased levels of newly synthesized

apoB. The siRNA did not affect apoB secretion, and it seemed to cause a decrease in release of apoB and ubiquitinated apoB into the cytosol.[33]

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Disclosures

None.

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