It has been well established that the biogenesis of apoB is mediated co-translationally by the cytosolic proteasome. Here, however, we investigated the role of both the cytosolic proteasome as well as non-proteasome-mediated degradation systems in the post-translational degradation of apoB. In pulse-chase labeling experiments, co-translational (0-h chase) apoB degradation in both intact and permeabilized cells was sensitive to proteasome inhibitors. Interestingly, turnover of apoB in intact cells over a 2-h chase was partially inhabitable by lactacystin, thus suggesting a role for the cytosolic proteasome in the post-translational degradation of apoB. In permeabilized cells, however, there was no post-translational protection of apoB by lactacystin. Further investigations of proteasomal activity in HepG2 cells revealed that, following permeabilization, there was a dramatic loss of the 20 S proteasomal subunits, and consequently the cells exhibited no detectable lactacystin-inhibitable activity. Thus, apoB fragmentation and the generation of the 70-kDa apoB degradation fragment, characteristic of permeabilized cells, continued to occur in these cells despite the absence of functional cytosolic proteasome. Similar results were observed when we used a derivative of lactacystin, clastolactacystin, which represents the active species of the inhibitor. Interestingly, however, the abundance of the 70-kDa fragment could be modulated by the microsomal triglyceride transfer protein inhibitor, BMS-197636, as well as by pretreatment of the permeabilized cells with dithiothreitol. These data thus suggest that although the cytosolic proteasome appears to be involved in the post-translational turnover of apoB in intact cells, the specific post-translational fragmentation of apoB generating the 70-kDa fragment observed in permeabilized cells occurs independent of the cytosolic proteasome.

Hepatic apolipoprotein B100 (apoB) secretion appears to be regulated post-transcriptionally (1–4). More specifically, efficient translocation of newly synthesized apoB molecules across the membrane of the endoplasmic reticulum (ER) is believed to be an important event that contributes to the formation of secretion competent apoB-containing lipoproteins (5–8). Inefficient translocation has been suggested to lead to the formation of a pool of membrane-associated apoB that becomes ubiquitinated (12–20, 22) and ultimately destined for intracellular degradation (6, 8–11). It is evident that co-translational degradation of membrane-associated apoB is mediated by the cytosolic proteasome based on its sensitivity to proteasome inhibitors such as ALLN, lactacystin, and MG132 (9, 12–22).

The involvement of the proteasome in degradation of apoB raises a number of intriguing questions. Clearly, the proteasome is involved in co-translational degradation of membrane-associated apoB, which is expected to have cytosolic exposure. However, recent evidence has suggested that the proteasome may also be involved in the post-translational degradation of apoB (19, 21, 22). Post-translational degradation of apoB in HepG2 cells has generally been thought to occur in the ER or a closely associated compartment (5, 16, 23, 24), although studies in rat hepatocytes have suggested that degradation of apoB may occur in post-ER compartments (25–27). It is speculated that a number of degradation systems may be involved in the intracellular turnover of apoB. Degradation of apoB appears to generate distinct proteolytic intermediates in both intact (9, 28) and permeabilized HepG2 cells (16). In permeabilized cells, apoB degradation occurs by a temperature- and pH-dependent and ALLN-sensitive cysteine protease in an ER-related compartment (16), resulting in the generation of an abundant N-terminal 70-kDa fragment, which can be detected in the lumen of the secretory pathway (16, 29, 30). In addition, we have reported that the apoB associated with luminal lipoproteins is also degraded post-translationally by an ALLN-sensitive degradation mechanism and could be rescued from degradation by cytosolic factors and metabolic energy, perhaps by inducing transport of apoB out of the degradation compartment (30). Furthermore, Wu et al. (14) have reported a two-site model for the degradation of apoB in HepG2 cells, suggesting that after the initial rapid degradation process, apoB that is fully translocated into the ER lumen can still undergo degradation via a second proteolytic system that is ALLN-resistant but sensitive to DTT.

In the present report, we used both intact and permeabilized HepG2 cell systems (16, 29, 30) to study the role of the cytosolic proteasome in post-translational apoB degradation. We report that post-translational degradation of apoB in intact cells is partially sensitive to proteasomal inhibitors, thus suggesting the involvement of the proteasome. Interestingly, however, we found that permeabilization of HepG2 cells results in the loss of proteasome function as determined by the loss of both lacta-

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**EXPERIMENTAL PROCEDURES**

**Materials**—HepG2 cells (ATCC HB 8065) were obtained from the American Type Culture Collection. Fetal bovine serum (certified grade) and cell culture media were from Life Technologies (Toronto). Digitonin of a higher purity (100%) was obtained from Calbiochem. Rabbit antisera to 20 S proteasome antibody was a kind gift from Walter Ward (Texas), and the microsomal triglyceride transfer protein (MTP) inhibitor BMS-197636 was provided by Dr. David Gordon (Bristol Meyers Squibb, Princeton, NJ). Lactacystin was purchased from Dr. E. J. Corey (Harvard University).

**Cell Culture**—Cultures of HepG2 were maintained in an α-modification of Eagle’s minimal essential medium (α-MEM) containing 10% fetal bovine serum. Cells were grown in 35-, 60-, or 100-mm dishes at 37 °C, 5% CO2 in complete medium (α-MEM, 10% fetal bovine serum) (31). Cultures were allowed to reach 75–80% confluence before experiments were carried out.

**Metabolic Labeling of Intact Cells, Permeabilization, and Determination of ApoB Degradation**—Nearly confluent HepG2 cultures were incubated with methionine/cysteine-free MEM for 120 min, pulse-labeled (15–120 min; see figure legends) with 100 μCi/ml 35S protein labeling mix (Pro-MixTM), washed three times, and chased for various chase periods (10–120 min) in culture medium supplemented with 10 mM methionine and 2 mM cysteine. At the end of each chase period, the medium was removed, and the cells were washed once with phosphate-buffered saline. The cells were harvested in a solubilization buffer (phosphate-buffered saline containing 1% Nonidet P-40, 1% deoxycholate, 5 mM EDTA, 1 mM EGTA, 1 mM phenylmethylsulfonyl fluoride, 100 kallikrein-inactivating units/ml Trasylol, 0.1 mM leupeptin, 0.5 mM ALLN). Cell extracts and media were centrifuged in a microcentrifuge at 14,000 rpm for 10 min, and the supernatant was subjected to immunoprecipitation.

To examine apoB degradation in permeabilized cells, intact cells chased for 10 min were washed and incubated for 10 min at room temperature in cytoskeletal (CSK) buffer (0.3 M sucrose, 0.1 M KCl, 2.5 mM MgCl2, 1 mM sodium-free EDTA, 10 mM PIPES, pH 6.8) containing 50 μM digitonin. Permeabilized cells were washed three times in cysteine-inhibitable proteasome activity as well as proteasomal subunits. Permeabilized cells that are largely devoid of the cytosolic proteasome appear to continue to degrade apoB, generating specific fragments, including the 70-kDa fragment, via a lactacystin-insensitive process. Hence, the permeabilized cell model provides a system to study post-translational apoB fragmentation in the absence of the functional cytosolic proteasome. Moreover, the data from the permeabilized cell system suggests that post-translational turnover of luminal apoB may also involve nonproteasomal degradation systems.

**FIG. 1.** Lactacystin affects co- and post-translational turnover of apoB in intact HepG2 cells. A, nearly confluent cells were pulsed for 15 min with 35S protein labeling mix, and the radioactivity was chased for 120 min. Lactacystin (LC; 25 μM) was added to some dishes 60 min before the pulse and was included during both pulse and chase periods. After the chase, medium was collected, and cells were solubilized. Cell lysates were immunoprecipitated with anti-apoB antibody, and the immunoprecipitates were analyzed by SDS-PAGE and fluorography. The percentage of apoB remaining after 60 and 120 min was assessed by determining the amount of radioactivity in the apoB100 bands (recovered from media and cell lysates of control and lactacystin-treated cells) as a percentage of the amount recovered at 0 chase time (n = 3). Lactacystin (25 μM) was present at all steps of the pulse-chase protocol including preincubation, pulse, and chase. Open circles, without lactacystin; closed circles, with lactacystin.

**FIG. 2.** In permeabilized HepG2 cells lactacystin inhibits co-translational turnover of apoB but does not block post-translational degradation of apoB or the generation of the 70-kDa fragment. A, nearly confluent HepG2 cells were pulsed for 15 min with 35S protein labeling mix, chased for 10 min, and permeabilized with digitonin, and permeabilized cells were then incubated in CSK buffer. In some dishes, lactacystin (25 μM) was included in the preincubation medium, the pulse, and the chase, as well as during the incubation of permeabilized cells in CSK buffer. Permeabilized cells were solubilized and then immunoprecipitated with a polyclonal anti-apoB antibody. Immunoprecipitates were analyzed by SDS-PAGE and fluorography. The arrowheads indicate the 550-kDa apoB100 and its 70 kDa degradation intermediate. B, for the experiment in A, the turnover of apoB in permeabilized cells in the presence and absence of lactacystin was assessed by plotting the total apoB radioactivity recovered (in the apoB100 bands) from permeabilized cells at various times of incubation in CSK buffer (zero time, 60 min, and 120 min). Lactacystin (LC) was present at all steps of the pulse-chase protocol including preincubation, pulse, and chase. Open circles, without lactacystin; closed circles, with lactacystin.
CSK buffer and were then incubated in CSK buffer under various conditions as described in the figure legends. The cells were harvested in solubilization buffer, and the cell extracts were subjected to immunoprecipitation.

**Microsomal Triglyceride Transfer Protein Inhibitor Studies**—Nearly confluent HepG2 cells grown in six-well plates were incubated for 1 h in methionine/cysteine-free medium containing 0–50 nM MTP inhibitor (BMS-197636) and 20 μg/ml ALLN. Following the preincubation, cells were briefly pulsed with 35S protein labeling mix and chased for 10 min in α-MEM plus 10 mM 1-methionine. Cells were washed three times in CSK buffer and permeabilized with digitonin (50 μg/ml, 10 min). At this point (0 time), control and treated cells were collected, and the remaining cells were incubated in CSK buffer supplemented with or without the MTP inhibitor. After the first hour of incubation, CSK buffer was removed, the cells were washed twice, and fresh CSK was added for the remaining 2 h. The cells were harvested in solubilization buffer, and the cell extracts were subjected to immunoprecipitation.

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**Fluorescein Isothiocyanate-labeled Casein Assay for Proteasome Activity**—Cells were incubated in complete α-MEM in the presence and absence of 25 μM lactacystin for 1 h. Some cells were then washed in CSK and permeabilized as described above, and other cells were left intact. Both intact and permeabilized cells were collected in phosphate-buffered saline in the presence of 0.1% Triton X-100 and homogenized in a Dounce glass homogenizer. Cell homogenates were centrifuged in a microcentrifuge at 14,000 rpm for 10 min, and the supernatant was subjected to protease assays. The protease assays were performed in 1.5-ml centrifuge tubes according to Twining (32) with modifications to assess proteasomal activity. To each reaction tube the following components were added: 20 μl of the respective sample, 25 μl of a 100 mM Tris, pH 7.8, buffer, 5 μl of 5 mg/ml fluorescein isothiocyanate-labeled protein, and 20 μl of 5 mg/ml fluorescein isothiocyanate-labeled casein. Following the incubation, the tubes were centrifuged, and the amount of solubilized casein was determined by fluorometry.

**Fig. 3.** Permeabilization of HepG2 cells does not cause leakage of radiolabeled apoB into the surrounding medium. Nearly confluent HepG2 cells were pulsed for 15 min with 35S protein labeling mix, chased for 10 min, and permeabilized with digitonin (50 μg/ml, 10 min), and permeabilized cells were incubated in CSK buffer for different times. Cells and CSK buffer were immunoprecipitated for apoB, and immunoprecipitates were analyzed by SDS-PAGE and fluorography. A, a representative experiment showing the distribution of apoB100 and the 70-kDa fragment in permeabilized cells and CSK buffer over a 2-h chase period; C, a second representative experiment showing the distribution of apoB in permeabilized cells and CSK buffer over a 3-h chase period (0, 1, 2, and 3 h). B and D, comparison of the amount of immunoprecipitable radiolabeled full-length apoB detected in permeabilized cells (closed circles) and the CSK buffer (open circles) for each incubation period (mean ± S.D.) from experiments in A and C, respectively.
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Fig. 4. Comparison of proteasomal activity in intact and permeabilized cells using a fluorogenic protease assay. Nearly confluent cells were preincubated in the presence or absence of lactacystin (25 μM) for 60 min. Some cells were then permeabilized, and others were left intact. All cells were then collected in phosphate-buffered saline plus 0.1% Triton X-100, homogenized 15× in a glass homogenizer, and centrifuged. Cell lysates were then used in performing fluorogenic protease assays according to modifications of Twining (29) with fluorescein isothiocyanate-labeled casein as the proteolytic substrate. Following the 1-h incubation time, samples were treated with 5% trichloroacetic acid to precipitate insoluble proteins and centrifuged. Then 60 μl of the supernatant was diluted with 400 μl of 500 mM Tris, pH 8.8, and read in a Fluoroskan fluorescent spectrophotometer at an excitation wavelength of 485 nm and emission wavelength of 538 nm. A comparison of total proteolytic activity between intact (open bars) and permeabilized cells (closed bars) (n = 4). B, comparison of lactacystin-inhibitable protease activity as assessed by the difference in fluorescent units between control and lactacystin-treated intact cells (open bars) and permeabilized cells (closed bars).

RESULTS

Effects of Lactacystin on the Co- and Post-translational Degradation of ApoB in Intact Cells—In an attempt to distinguish the role of the proteasome in co- and post-translational degradation of apoB, intact HepG2 cells were first pretreated with the proteasome inhibitor, lactacystin, and then pulsed and chased over a 120-min period. Lactacystin pretreatment of cells 30 min before the pulse induced a significant increase in the amount of apoB accumulated at 0 h (2.6-fold increase over control), which remained high during the 120-min chase (Fig. 1A). To assess post-translational sensitivity to lactacystin, cells were chased for 60 and 120 min in the presence and absence of the inhibitor (Fig. 1B). In control cells, 53% of apoB was recovered after a 60-min chase, whereas in lactacystin-treated cells 78% was recovered. This difference was also observed after 120 min of chase time with only a 48% recovery of apoB in control cells as opposed to a 76% recovery in lactacystin-treated cells. Hence, even after a 2-h chase, degradation of apoB remained sensitive to lactacystin, thus suggesting a role for the proteasome after translation of apoB. Nevertheless, there was still an approximate 24% loss in 35S-labeled apoB during the chase of lactacystin-treated cells, despite the presence of the inhibitor (Fig. 1B).

Effect of Lactacystin on Post-translational Fragmentation of ApoB in Permeabilized Cells—We used a permeabilized cell system in conjunction with lactacystin to further examine the role of the proteasome in the specific fragmentation of apoB and the generation of the 70-kDa degradation fragment. Intact HepG2 cells were briefly pulsed and chased and then permeabilized with 50 μg/ml digitonin. Under these conditions, per-
meabilized cells do not possess protein synthesis activity nor secrete proteins, but they retain the capability to intracellularly degrade proteins including apoB. To monitor degradation of the newly synthesized radiolabeled apoB pool, permeabilized cells were incubated in CSK buffer with and without lactacystin for 120 min. The inhibitory function of lactacystin demonstrates a lag period (34); thus, the addition of this drug 30 min before the pulse was necessary to ensure that the inhibitor was present in its active form during the pulse. Fig. 2, A and B, shows that a significantly higher level of apoB was present in lactacystin-treated permeabilized cells at zero time, in comparison with cells not treated with lactacystin (3.4-fold increase over control). Over the 120-min chase following permeabilization, a major proportion of 35S-labeled apoB100 was degraded in both control and lactacystin-pretreated cells (Fig. 2B). Furthermore, in the presence of lactacystin, there was a dramatic accumulation of the major degradation intermediates of apoB, including the 70-kDa fragment (Fig. 2A). Enhanced generation of the fragment appeared to result from a greater pool of 35S-labeled apoB100 at zero time in lactacystin-treated cells.

We also examined the possibility that the digitonin treatment of the cells may have resulted in the leakage of apoB from the secretory pathway (ER-Golgi system) into the CSK buffer. After permeabilization, we collected both the cells and CSK buffer at 0 h (CSK plus digitonin) and at different periods of chase and probed for apoB. Two representative experiments are shown. The amount of intact apoB in permeabilized cells declined over the chase period, concomitant with an increase in the accumulation of the 70-kDa fragment in the cells (Fig. 3). Little or no radiolabeled full-length apoB or the 70-kDa fragment could be detected in the CSK buffer at different chase times, and the amount detected was negligible compared with total immunoprecipitable apoB (Fig. 3). There was also no continued accumulation of the intact apoB or its 70-kDa fragment in the CSK buffer over time. Overall, the data suggest that there is minimal nonspecific loss of radiolabeled apoB following permeabilization of HepG2 cells and that temporal disappearance of intact apoB in permeabilized cells can be accounted for by intracellular degradation rather than cell leakage.

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**Comparison of Proteasomal Activity in both Intact and Permeabilized Cells**—In order to assess any effects permeabilization may have on the proteasomal activity of HepG2 cells, we measured protease activity of cell lysates prepared from intact and permeabilized HepG2 cells that were pretreated with and without lactacystin. In this particular assay, all detectable proteolytic activity was measured using fluorescein isothiocyanate-labeled casein as the proteolytic substrate. We initially examined the difference in total proteolytic activity between intact and permeabilized cells without lactacystin treatment and found that there was a decrease in the amount of proteolytic activity after permeabilization of the cells (0.23 ± 0.16 fluorescent units detected in permeabilized cells in comparison with 5.97 ± 1.2 fluorescent units detected in intact cells) (Fig. 4A). In the presence of lactacystin, there was a considerable
decrease in detectable proteolytic activity in intact cells (lactacystin-inhibitable activity, 2.84 ± 0.75 fluorescent units) (Fig. 4B). The loss in proteolytic activity was considered to be proteasomal in nature, since the difference between the two conditions was the presence of the proteasomal inhibitor, lactacystin. We then examined permeabilized cells and found no detectable lactacystin-inhibitable proteolytic activity (Fig. 4B).

The absence of any appreciable lactacystin-inhibitable protease activity in permeabilized cells suggests significant loss of proteasomal activity in these cells.

Detection of the 20 S Proteasomal Subunits in Intact and Permeabilized Cells—To assess whether the loss of proteasomal activity following permeabilization was due to the loss of proteasome subunits, we immunoblotted intact and permeabilized cells for several of the subunits of the 20 S proteasome that have both structural and functional roles in the proteasome complex. Immunoblotting of intact HepG2 cell lysates with the polyclonal antibody revealed several of the 20 S subunits ranging in size from 25 to 35 kDa (Fig. 5A). However, immunoblotting of permeabilized cell lysates revealed a dramatic reduction in the detection of the 20 S subunits (~68% reduction compared with intact cells), thus indicating a significant loss of the cytosolic proteasome upon permeabilization (Fig. 5B). Furthermore, a significant amount of proteasomal subunits was detected in CSK plus digitonin buffer used in the permeabilization of the cells (~48% detected in comparison with intact cells) (Fig. 5B).

Differential Sensitivity of ApoB Degradation in Permeabilized Cells to Lactacystin, Clastolactacystin β-Lactone, and ALLN—We compared the inhibitory effects of ALLN and lactacystin on post-translational degradation of apoB in permeabilized cells. In contrast to lactacystin, ALLN increased the amount of apoB remaining after a 2-h chase (Fig. 6A). The apoB remaining (as a percentage of total apoB at time 0) in lactacystin-treated and ALLN-treated permeabilized cells was 30.7 ± 5.2% and 75.1 ± 6.7%, respectively (p < 0.05, n = 3). Thus, unlike lactacystin, ALLN appeared to inhibit post-translational apoB degradation. In addition, ALLN also inhibited the post-translational apoB fragmentation, which generates the distinct apoB intermediates in permeabilized cells, including the 70-kDa fragment (Fig. 6B).

We also tested the inhibitory effect of clastolactacystin β-lactone, the active species of the lactacystin inhibitor (34), to ensure that the insensitivity of apoB fragmentation to lactacystin was not a result of the inability of the inhibitor to convert to its active form in a permeabilized cell. The addition of clastolactacystin β-lactone either before or after permeabilization did not prevent the loss of apoB in permeabilized cells, nor did...
it interfere with the appearance of the 70-kDa fragment (Fig. 6C). Interestingly, the addition of the inhibitor before the pulse resulted in an increase in the abundance of the 70-kDa fragment, most likely as a result of an increased initial pool of the full-length apoB from a diminished co-translational degradation (Fig. 6C).

**Effect of MTP Inhibition and DTT on the Degradation of ApoB in Permeabilized HepG2 Cells**—In an attempt to further characterize the degradation of apoB in permeabilized HepG2 cells, we used an inhibitor of MTP. Fig. 7A shows the immunoprecipitated apoB isolated from permeabilized HepG2 cells following 0 and 2 h in CSK with and without the MTP inhibitor. In control cells, the majority of apoB present at 0 h is degraded after the 2-h incubation in CSK (~83% degradation) and is accompanied by the generation of the 70-kDa apoB fragment. Quite surprisingly, less degradation of apoB was observed in MTP inhibitor-treated cells after a 2-h incubation (~45% degradation) in comparison with control cells. The amount of intact apoB remaining after 2 h was significantly higher in cells treated with the inhibitor (Fig. 7B) despite a considerable decrease in the amount of apoB present at 0 h in MTP inhibitor-treated cells. In addition, although not statistically significant, the generation of the 70-kDa fragment was also reduced in MTP inhibitor-treated cells (Fig. 7C).

In order to confirm the above observations, the experiment in permeabilized cells was conducted using concentrations of the MTP inhibitor ranging from 1 to 50 nM. Fig. 8A shows the percentage of apoB remaining in control cells and MTP inhibitor-treated cells following a 2-h incubation period. There was no significant difference between the percentage of apoB remaining in control cells and cells treated with the lowest concentration (1 nM) of the inhibitor; however, with increasing concentrations of the inhibitor (10 and 50 nM), the percentage of apoB remaining also increased (Fig. 8A). An inverse relationship was observed between the level of inhibition of MTP and the susceptibility of apoB to fragmentation in permeabilized cells, with the greatest amount of 70-kDa fragment detected in control cells (Fig. 8D).

The reducing agent DTT was also employed to examine whether or not modulation of the redox state of the ER could affect the fragmentation of apoB in permeabilized cells. Fig. 9 shows the alteration in the apoB fragmentation pattern observed in DTT-treated cells compared with control cells. ApoB degradation occurred in DTT-treated without the generation of the 70-kDa apoB fragment.

**DISCUSSION**

Intracellular degradation of apoB is very active in HepG2 cells, resulting in the secretion of only a small fraction of the total apoB synthesized. The intracellular mechanisms responsible for degradation of nascent apoB chains have been the subject of intense investigation in the past few years (5, 6, 9, 30, 35). Many recent reports have shown evidence for the involvement of the proteasome in the co-translational degradation of apoB in HepG2 cells (12–22). This evidence includes the sensitivity of apoB degradation to various proteasome inhibitors (12, 13, 15, 18–22), the detection of ubiquitinated apoB (12, 18–20, 22), and more recently the association of ubiquitinated apoB with the Sec61 complex of the translocon (18, 19). Our data in the present report suggest the involvement of the cytosolic proteasome not only in co-translational apoB degradation but also in its post-translational turnover. Recent reports (19, 22) have also provided evidence for the involvement of the cytosolic proteasome in the post-translational degradation of apoB. Laio et al. (22) have suggested that following translation and translocation in the ER, apoB may be targeted for proteasome-mediated degradation via retrograde transloc-
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To further characterize the degradative process operating in permeabilized cells, we examined the effects of inhibiting MTP on the generation of the 70-kDa fragment in permeabilized cells. Since inhibition of MTP increases the pool of secretion-incompetent apoB, we explored the role of this alternate degradation system on this pool of apoB (30). The results established an inverse relationship between the concentration of the MTP inhibitor and the generation of the 70-kDa fragment, thus suggesting that the pool of apoB accumulated in the presence of MTP inhibitor was not accessible to the degradation machinery responsible for generation of the fragment. Instead, it seems likely that a large percentage of this pool of apoB is destined for co-translational degradation via the proteasome on the cytosolic side of the ER membrane. This hypothesis is also in accordance with the findings of Benoist and Grand-Perret (15), suggesting that inhibition of MTP activity may induce co-translational degradation of apoB by the proteasome. This in turn would suggest that less apoB is localized to the ER lumen, thus leading to a decrease in the generation of the 70-kDa fragment.

Studies were also performed with the reducing agent DTT, and results revealed that in the presence of DTT there was an accelerated in the post-translational degradation of apoB in permeabilized HepG2 cells. Previous work in our laboratory (44) as well as others (45) has shown that disruption of disulfide bond formation within the apoB molecule can inhibit the secretion of apoB and induce its intracellular degradation. The observation that apoB degradation in DTT-treated cells occurred without the generation of the 70-kDa apoB fragment suggests that the degradation pathway may be altered in the presence of DTT. We hypothesize that the presence of DTT induces conformational changes in apoB and stimulates its rapid co-translational degradation, thus not allowing for delivery of full-length substrate to a second degradative pathway.

There is precedence for the involvement of proteases functioning in conjunction with the proteasome in the regulated degradation of proteins in eukaryotic cells (46, 47). Our data suggest that in intact cells, if newly synthesized apoB chains are rescued from co-translational proteasome-mediated degradation, they are still sensitive to the proteasome following translation and translocation (based on data in intact cells) and other nonproteasomal degradative system(s) (based on data in permeabilized and intact cells). It is likely that there is an association between proteasomal and nonproteasomal degradation systems. This is particularly apparent from our observation that inhibition of co-translational proteasomal degradation by pretreatment with lactacystin increases the abundance of the 70-kDa fragment apparently by providing more substrate for the post-translational non-proteasome-mediated fragmentation process. Hence, further studies are clearly needed to elucidate the complex interrelationship between proteasomal and nonproteasomal degradative systems that act to destabilize the secretion incompetent apoB intracellularly.

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