We studied the effect of inhibition of microsomal triglyceride transfer protein (MTP) on apolipoprotein (apo) B100 translation and secretion using HepG2 cells. The MTP-mediated lipid transfer activity was reduced using a specific MTP inhibitor. ApoB100 translation was synchronized by treatment with puromycin prior to L-[35S]methionine pulse-chase labeling. During the first 4 min of chase, synthesis of apoB polypeptides the size of 100–200 kDa was insensitive to the inhibitor, suggesting that inhibition of MTP did not affect the initiation of apoB100 translation. After 15 min of chase, the 100–200-kDa species were chased into polypeptides larger than 320 kDa (i.e. apoB65 or 65% of full-length apoB100) in both control and inhibitor-treated cells. However, the amount of these polypeptides decreased (by 36% for apoB65-75, by 64% for apoB75-85, by 76% for apoB85-95, and by 77% for apoB100) upon MTP inhibition. No accumulation of smaller polypeptides was observed, but total immunoprecipitable apoB radioactivity was decreased suggesting that apoB could undergo co-translational degradation when MTP activity was reduced. Inhibitors of the multicatalytic proteinase complex (proteasome) such as lactacystin or MG-115 could prevent apoB co-translational degradation. Nevertheless, MG-115 could not avoid the MTP inhibitor decreasing apoB100 secretion but rather induced the accumulation of secretion-incompetent apoB100 in the cell. These results indicate that MTP activity is required during the elongation of apoB100 polypeptides, particularly at the sequences downstream of carboxyl terminus of apoB65. Co-translational degradation might constitute a more general mechanism of early quality control for large or complex proteins.

Association with lipids is a prerequisite for the secretion of apolipoprotein (apo) B100, a structural protein of low density lipoprotein, from hepatocytes. Microsomal triglyceride transfer protein (MTP), a heterodimeric protein containing a 97-kDa subunit and protein disulfide isomerase (1, 2), may facilitate the assembly of lipids and apoB in the lumen of endoplasmic reticulum (ER). Using cells transfected with cDNAs encoding the 97-kDa subunit of MTP or truncated forms of human apoB, several laboratories have demonstrated that MTP is necessary and sufficient for apoB secretion as a lipoprotein (3–6). This is consistent with the genetic evidence that defective MTP is the cause of abetalipoproteinemia (7–10).

The sequence of events that lead to the assembly of hepatic very low density lipoprotein (VLDL)-containing apoB has been progressively unraveled. In rat liver cells, assembly of VLDL-containing apoB48 (the amino terminus 48% of apoB100) is most likely achieved through two discrete steps (11, 12). Accumulating results obtained from studies using different cellular models (13–16) have indicated that during the first step of assembly apoB is lipidated co-translationally and is translocated across the ER membrane. The lipid content of the primordial lipoproteins is directly related to the length of apoB polypeptides (13, 15, 16). It is now clear that MTP plays an obligatory role in the initial stage of apoB lipidation (3–6, 17, 18). However, whether or not apoB translocation across the ER membrane is tightly coupled with translation remains controversial (19–21). In the second step, the primordial lipoprotein particles acquire additional neutral lipids to form a mature VLDL (11, 12). Unlike the first step lipidation, the recruitment of lipids during the second step apoB48-VLDL formation is not governed by the length of apoB but seems mediated by short hydrophobic sequences within apoB48 (22). In the rat hepatoma McA-Rh7777 cells, the second step lipidation of rat apoB48 does not seem to require the activity of MTP (17).

In liver cells, a significant amount of newly synthesized apoB100 is degraded prior to secretion (23). Early studies showed that this presecretory degradation took place at the post-translational level (23–25), and a variety of factors can modulate apoB100 secretion efficiency by altering the ratio of degraded versus secreted apoB100 (26–29). Among others, the availability of lipids is the most important factor regulating the efficiency of apoB100 secretion (30–33). The cysteine protease inhibitor, N-acetyl-leucyl-leucyl-norleucinal (ALLN), could block the post-translational degradation of apoB (34, 35). ALLN has been shown to inhibit the proteasome, calpains and cathepsin B (36, 37). Recently, a kinetically and biochemically distinct pathway for the presecretory degradation of apoB100 has been suggested (5, 38–41). This very early degradation of apoB seems to occur during polypeptide elongation (i.e. co-translational) and before the translation of full-length apoB is...
completed. This putative co-translational proteolysis of apoB has been suggested to result from impaired translocation across the ER membrane and appears to be attenuated by expression of active MTP but is insensitive to ALLN (5). A physical interaction between MTP and apoB100 at the early stages of lipoprotein assembly have been demonstrated (42). Therefore, MTP may conceivably act as a molecular chaperone by facilitating translocation and proper folding of apoB via its lipid transmigrating activity. Using HepG2 cells treated with a potent and specific MTP inhibitor 4′-bromo-3′-methylmetaqualone, we have observed a decrease in the secretion of apoB100 and neutral lipids associated with lipoproteins (41). The decreased secretion of apoB and lipids by the MTP inhibitor has been suggested to reflect mainly an early presecretory degradation of apoB100 (41). We hypothesize that the apparent presecretory degradation of apoB upon MTP inhibition could be attributable to alteration of apoB polypeptide elongation.

In the current study, we attempted to determine if the lipid transfer activity of MTP was required for apoB100 translation in HepG2 cells. To delineate what is co-translational and what is post-translational, we have synchronized the polysomes. We have shown that inactivation of MTP with the inhibitor results in co-translational degradation of apoB100. This phenomenon becomes manifest only after the nascent polypeptide has reached 65% of the full-length apoB100 (i.e. apoB65) but can be prevented by proteasome inhibitors. Thus, by preventing apoB co-translational degradation, MTP is required for the elongation of apoB polypeptides, particularly of the sequences downstream of the carboxyl terminus of apoB65.

EXPERIMENTAL PROCEDURES

Materials—L-[35S]Methionine (37 TBq/mmol) was purchased from NEN Life Science Products. Basal medium Eagle’s, RPMI 1640 medium, t-glutamine, penicillin, streptomycin, and fetal calf serum were obtained from NEN Life Science Products. Basal medium Eagle’s, RPMI 1640 medium, t-glutamine, penicillin, streptomycin, and fetal calf serum were obtained from Life Technologies, Inc. ALLN and placental RNase inhibitor—were obtained from Boehringer Mannheim. Lactacycin was a generous gift of Dr. S. Obara (Tokyo, Japan). N-Acetyl-aspartyl-glutamyl-valyl-aspartyl was from Calbiochem and N-benzoxycarbonyl-tyrosyl-lysyl-arginyl-valyl-alanyl-aspartyl-fluoromethyl from Kamiya Biomedical Co. The MTP inhibitor 4′-bromo-3′-methylmetaqualone was synthesized by Glaxo Wellcome. Molecular weight markers for electrophoresis were Rainbow™ (Amersham Corp.) and apoB forms secreted by primary rat hepatocytes (apoB48, apoB65, and apoB100). All other chemicals were obtained from Sigma.

Cell Culture—HepG2 cell line was obtained from the American Type Culture Collection. The cells were seeded into 24-well plates (200,000 cells per well) containing basal medium Eagle’s supplemented with 10% fetal calf serum and were obtained from Life Technologies, Inc. ALLN and placental RNase inhibitor were obtained from Boehringer Mannheim. Lactacycin was a generous gift of Dr. S. Obara (Tokyo, Japan). N-Acetyl-aspartyl-glutamyl-valyl-aspartyl was from Calbiochem and N-benzoxycarbonyl-tyrosyl-lysyl-arginyl-valyl-alanyl-aspartyl-fluoromethyl from Kamiya Biomedical Co. The MTP inhibitor 4′-bromo-3′-methylmetaqualone was synthesized by Glaxo Wellcome. Molecular weight markers for electrophoresis were Rainbow™ (Amersham Corp.) and apoB forms secreted by primary rat hepatocytes (apoB48, apoB65, and apoB100). All other chemicals were from Sigma.

RESULTS

Fast Inhibition of ApoB100 Production by an MTP Inhibitor—Previously we demonstrated that 4′-bromo-3′-methylmetaqualone specifically inhibited MTP-mediated lipid transfer (41). This compound inhibited secretion of apoB100 in a dose-dependent manner but had no effect on secretion of other proteins from HepG2 or primary human hepatocytes (41). As shown in Fig. 1A, pulse-chase experiments with L-[35S]methionine revealed that the MTP inhibitor rapidly exerted its inhibitory effect on apoB secretion in HepG2 cells. When added 5 min prior to pulse (10 min duration) and chase (180 min duration), the inhibitor decreased secretion of apoB100 by 70% compared with control (Fig. 1A, lane b versus lane a). Decreased secretion of apoB100 could also be observed if the inhibitor was added immediately at the end of pulse (Fig. 1A, lane c). However, if the inhibitor was added 30 min after chase, apoB100 secretion was unaffected (Fig. 1A, lane d). These results suggest that the MTP activity is probably required during very early stages of apoB production.

To determine if the MTP inhibitor affects apoB100 secretion because of intracellular retention, we have also quantified apoB intracellular content. HepG2 cells were pulse-labeled for 10 min with L-[35S]methionine followed by 150 min of chase. Secreted and intracellular apoB100 were immunoprecipitated either in control cells or in cells treated with the MTP inhibitor added 2 min before the pulse. As shown in Fig. 1A, inhibition of MTP decreases both secreted apoB100 and the intracellular content (lanes f versus e). These results suggest that the MTP inhibitor either reduces apoB100 synthesis or increases apoB100 degradation. Thus, the results indicate the intracellular content, HepG2 cells were pulse-labeled for 15 min with L-[35S]methionine, and cells were lysed immediately at the end of the pulse (no chase). The level of intracellular apoB100 was decreased (by 65%) if the MTP inhibitor was added 5 min prior to pulse labeling, as determined by immunoprecipitation of the radiolabeled apoB (Fig. 1B, lanes k and l). A similar decrease is observed using the whole cell lysate (Fig. 1B, lanes i and j). The
Co-translational Degradation of ApoB100 Is Prevented by MTP

Fig. 1. The effect of MTP inactivation on apoB100 secretion and intracellular content. A, control HepG2 cells (lane a) were pulse-labeled with L-[35S]methionine for 10 min followed by 180 min of chase. The MTP inhibitor (5 μM) was added either 5 min before pulse (lane b) or else added 0 or 30 min after the beginning of chase (lanes c and d). Proteins secreted into the medium were quantified after SDS-PAGE by PhosphorImager screen autoradiography. Alternatively, HepG2 cells were pulse-labeled for 10 min followed by 180 min of chase and secreted or intracellular apoB100 were quantified after immunoprecipitation (lanes e–g and h–j, respectively). Control condition (lanes e and g) was compared with cells treated with the MTP inhibitor (5 μM) added 2 min before the pulse (lanes f and h). B, left, intracellular apoB100 was determined immediately after 15 min pulse labeling. Cell lysate (lanes i and j) or immunoprecipitated apoB100 (lanes k and l) was analyzed by SDS-PAGE. Control cells (lanes i and k) were compared with cells treated with 10 μM MTP inhibitor added 5 min before pulse (lanes j and l). Right, in vitro translation of apoB100 was performed at 30 °C for 90 min using HepG2 cell lysate as a source of mRNA (see “Experimental Procedures”). ApoB100 was immunoprecipitated and analyzed by SDS-PAGE followed by PhosphorImager screen autoradiography. Control condition (lane m) was compared with in vitro translation performed in the presence of 10 μM MTP inhibitor (lane n).

latter experiment ruled out the possibility that the MTP inhibitor might interfere with the yield of immunoprecipitation of apoB. Since the radioactivity associated with apoB100 was determined immediately after 15 min of pulse, the decreased intracellular apoB100 by inactivation of MTP might reflect reduced apoB100 synthesis. Pretreatment of the cells for 60 min with 10 μM actinomycin D, which decreased by 97% messenger RNA synthesis, did not change the basal apoB100 secretion nor the effect of the MTP inhibitor (data not shown), suggesting that there was little alteration at the transcriptional level. When apoB100 translation was determined in vitro using HepG2 cell lysate (Fig. 1B, lanes n and m), we found that MTP inhibitor (10 μM) did not affect apoB100 translation (99 ± 13% of control). These data indicate that the MTP inhibitor had no direct effect on the translation machinery. They also suggest that apoB100 production could be regulated by MTP activity in intact cells during very early stages, possibly at the co-translational level.

Synchronization of ApoB100 Translation in Living Cells—We determined the elongation rate of apoB polypeptides by pulse-chase experiments with radiolabeled amino acids. The chase time at which incorporation of radioactivity into the full-length protein reached the maximum represented the time required for translation of apoB100. With HepG2 cells, we found that about 20 min were required for the entire apoB100 polypeptide to be synthesized (data not shown). However, the lack of synchronization of translation in the cells renders difficult the monitoring of apoB elongation at the early stages of translation. Even with a very short period of pulse (2 min), apoB polypeptides of various lengths (ranging from 70 to 514 kDa) representing nascent chains at different stages of maturation were labeled as shown in Fig. 2 (lane a) and as reported previously (46). If the pulse is followed by a chase, full-length apoB100 accumulates without the detection of low molecular mass nascent polypeptides (Fig. 2, lane b). This indicates that the labeled methionine randomly incorporated along the polypeptide chain during the pulse period is chased into large and full-length polypeptides. In such conditions it is difficult to delineate what is co-translational and what is post-translational. To observe apoB polypeptide elongation, we developed a protocol to synchronize translation of the apoB mRNA by treating the cells with puromycin prior to the pulse-chase labeling with L-[35S]methionine (see “Experimental Procedures”). Puromycin interferes with tRNA binding to ribosome and induces the premature release of polypeptides from the ribosome (13, 15, 47). When cells were pretreated with puromycin (10 μM) and pulse-labeled 5 min prior to a 10-min chase, only 350–450-kDa polypeptides could be detected (Fig. 2, lane c). Since no full-length apoB100 could be detected, it indicates...
that nascent polypeptides were released by the puromycin pretreatment and that translation restarted from the amino-terminal extremity after puromycin removal. The smallest radiolabeled polypeptide has the same size with or without puromycin pretreatment (Fig. 2, lane c versus lane b) indicating that translation restarts without lag time (except for puromycin concentration above 50 μM, data not shown). Puromycin pretreatment of the cells decreases total apoB labeling probably because the radiolabeled methionine could only be incorporated into the amino-terminal part of apoB100 instead of being incorporated all along the sequence.

**Effect of the MTP Inhibitor on ApoB100 Polypeptide Elongation**—To study the role of MTP on apoB polypeptide elongation, HepG2 cells were pretreated with 10 μM puromycin prior to pulse-chase with or without 5 μM MTP inhibitor. Incorporation of radioactivity into apoB polypeptides of 100–200 kDa was identical between control and the inhibitor-treated cells at the end of 4 min of chase (Fig. 3A, lanes a and b), indicating that partial inactivation of MTP did not affect the initiation of apoB100 translation. However, conversion of the 100–200-kDa species into higher molecular mass polypeptides (Fig. 3A, lanes c and d, 15 min chase) as well as the full-length apoB100 (Fig. 3A, lanes e and f, 35 min chase) was markedly decreased by inactivation of MTP. Since the decrease in high molecular mass polypeptides could be seen within 15 min of chase (i.e. before the entire apoB100 was translated), these results indicate that MTP inhibitor alters apoB100 production at the co-translational level. During the entire chase, no accumulation of low molecular mass polypeptides was observed nor was there any alteration in the size of apoB polypeptides by inactivation of MTP.

**FIG. 3.** The effect of MTP inactivation on apoB polypeptide elongation. A, left, HepG2 cells were treated with puromycin (10 μM) for 5 min at 37 °C and washed as in Fig. 2 before pulse labeling with L-[35S]methionine for 5 min at 35 °C and chased for 4 min (lanes a and b), 15 min (lanes c and d), and 35 min (lanes e and f), respectively, before cell lysis and immunoprecipitation of apoB. After SDS-PAGE, radioactivity associated with apoB was quantified using a PhosphorImager screen. Puromycin-treated cells in control condition (lanes a, c, and e) were compared with cells also treated with 5 μM MTP inhibitor (lanes b, d, and f). The MTP inhibitor was added together with puromycin and maintained through entire washings, pulse and chase. Right, apoB100 secreted as lipoproteins were isolated by ultracentrifugal flotation at d < 1.21 g/ml (lanes g and h). Control cells were compared with cells treated with 5 μM MTP inhibitor added at the beginning of the 30-min pulse labeling and maintained through the 180-min chase. B, intracellular apoB polypeptides were immunoprecipitated after 15 min pulse with L-[35S]methionine as in Fig. 1B, lanes k and l. Control condition (solid line) was compared with cells treated with 10 μM MTP inhibitor added 5 min before the pulse (dotted line). The area under the curve for apoB70–95 in the presence of MTP inhibitor was shown in black. A. U., arbitrary units.
MTP (Fig. 3A, lanes a–f). Thus, inhibition of MTP does not alter the rate of apoB polypeptide elongation per se, nor does it cause any discernible pause or arrest of apoB translation. In both control or the inhibitor-treated cells, only the full-length apoB100 was secreted as a lipoprotein (Fig. 3A, lanes g and h). As expected, secretion of apoB100 incorporated into lipoproteins (at the end of 3 h chase) was decreased from cells treated with the MTP inhibitor.

Quantification by scanning the phosphor screen autoradiograph (Fig. 3A) revealed that the amount of radioactivity associated with total apoB polypeptides was decreased by 40 and 55%, respectively, at the end of 15 and 35 min chase by MTP inhibition as compared with control (Table I). In addition, loss of radioactivity associated with total apoB polypeptides during chase was observed in both control (by 60%) and the inhibitor-treated (by 82%) cells (Table I, total apoB between 4 and 35 min chase). This result is consistent with the previously described post-translational degradation of apoB (23–25). Moreover, the incorporated radioactivity was decreased by the MTP inhibitor even before apoB100 had reached its full length (15 min chase), indicating that apoB100 degradation occurs at least in part at the co-translational level.

To elucidate further the effect of MTP inactivation on apoB100 co-translational degradation, we quantified radioactivity associated with apoB polypeptides of different sizes (Table I). While the amount of polypeptides resembling the size of apoB55-65 (i.e., from 55 to 65% of the full-length apoB100) was almost not affected by the MTP inhibitor, the amount of larger polypeptides (e.g., apoB65-75, apoB75-85, and apoB85-95) was decreased progressively in relation to their size. The difference of effect of the MTP inhibitor between large and small polypeptides was not due to slow entry of the inhibitor into the ER lumen, since similar results were obtained when cells were incubated with the inhibitor 1 h prior to the addition of puromycin (data not shown). The decrease in the amount of radioactivity associated with polypeptides the size of apoB55-65 by MTP inactivation was similar to that with the full-length apoB100 after 35 min of chase (Table). These results suggest that inactivation of MTP enhances degradation of nascent apoB polypeptides during chain elongation mainly at chain length between 65 and 85% of the full-length apoB100. The possibility that the co-translational degradation is a consequence of polysome synchronization can be ruled out since the MTP inhibitor also decreases the amount of high molecular mass apoB polypeptides when a simple pulse protocol is used (Fig. 3B).

The Proteasome Is Involved in ApoB100 Co-translational Degradation—Post-translational degradation of apoB100 can be inhibited by the cysteine protease inhibitor ALLN (34, 35, 48). Unfortunately, ALLN is poorly specific and inhibits several cysteine proteases including calpains, cathepsin B, and the proteasome (36, 37). We have previously shown that the reducing agent dithiothreitol could prevent the early degradation of apoB100 observed in cells treated with the MTP inhibitor (41). To better characterize the protease involved in the co-translational degradation of apoB100, we have screened several protease inhibitors. Because the synchronization protocol is not convenient for a screening, we have used the protocol of 15 min of pulse without chase as in Fig. 3B. Full-length apoB100 as well as nascent polypeptide the sizes of apoB70–95 and apoB20–65 have been quantified as in Table I. With such a protocol, the decreased level of apoB100 observed in the presence of the MTP inhibitor is mainly the reflect of the co-translational degradation since most of the post-translational degradation occurs later even in the presence of the MTP inhibitor (41). As shown in Fig. 4, only ALLN, MG-115, and lactacystin are able to prevent apoB100 co-translational degradation in MTP inhibitor-treated cells. Trans-Epoxyoxycinnaryl-leucylamido-(4-guanidino)butane, an inhibitor of calpains and cathepsin B (49, 50), cannot prevent apoB degradation. MG-115 and ALLN are inhibitors of calpains and cathepsin B but they can also inhibit the proteasome (36). At a lower concentration (26 μM), ALLN failed to prevent apoB co-translational degradation (data not shown). The concentration of ALLN (104 μM) or MG-115 (10 μM) required to prevent apoB degradation is consistent with the relative potency of ALLN and MG-115 to inhibit the proteasome (36). The involvement of the proteasome is confirmed by the effect of lactacystin, a specific proteasome inhibitor (51) of microbial origin (52).

Using the synchronization protocol, we confirm that 10 μM MG-115 can prevent the co-translational degradation of apoB70-95 polypeptides observed in the presence of the MTP inhibitor (Fig. 5).

Prevention of Co-translational Degradation Allows the Synthesis of Secretion-incompetent ApoB100 in Cells Treated with MTP Inhibitor—We have studied the fate of apoB100 in the presence of a potent proteasome inhibitor. HepG2 cells treated with 10 μM MG-115 and with or without MTP inhibitor were pulse-labeled for 15 min with L-[35S]methionine. After 150 min of chase, secreted and intracellular apoB100 were determined by immunoprecipitation. Although apoB100 co-translational degradation is prevented by MG-115 (Figs. 4 and 5), the MTP inhibitor is still able to decrease the secretion of apoB100 (Fig. 6, top). The apoB100 intracellular content remains high even in the presence of 5 or 20 μM MTP inhibitor (Fig. 6, bottom). The percentage of apoB100 that is secreted after 150 min of chase is 45 ± 5% in MG-115 control condition but it falls down to 10 ± 2% and 7 ± 2% in the presence of 5 and 20 μM MTP inhibitor, respectively. This decrease in the secretion efficiency indicates that apoB100, having escaped co-translational degradation because of the proteasome inhibitor, is secretion incompetent when MTP is inhibited.
DISCUSSION

We have studied the effect of MTP inactivation on the translation of apoB100 using HepG2 cells treated with an MTP inhibitor. To monitor elongation of nascent apoB polypeptides, we have synchronized translation of apoB mRNA by treating the cells with puromycin prior to metabolic labeling with \(^{35}\)S-methionine. By this means, elongation of a relatively homogeneous population of apoB polypeptides could be observed in the chase period. Results presented herein demonstrate that inactivation of MTP decreases the number of full-length apoB100 polypeptides produced. This apparent decrease does not seem to be the consequence of a direct inhibition of apoB translation, since both in vitro translation assay (Fig. 1B) and metabolic labeling experiment using intact cells (Fig. 3A, lane a and b) have indicated that the initiation of apoB translation is insensitive to the MTP inhibitor or MTP inactivation. Rather, the decreased synthesis of full-length apoB100 appears to be the result of co-translational degradation when MTP is inactivated (Fig. 3A, lanes c–f and Table I). The ability of proteasome inhibitors to prevent the effect of the MTP inhibitor on apoB synthesis (Fig. 4) confirms that a true degradation process is involved. This also rules out the possibility that inhibition of MTP alters the elongation process per se. ApoB nascent polypeptides may be degraded proteolytically at multiple sites yielding fragmented products that become undetectable. Other authors have also suggested that apoB could undergo co-translational degradation (5, 39). The co-translational degradation of apoB can also be detected without synchronizing translation (Fig. 1B and Fig. 3B), confirming its physiologic relevance. However, the synchronization protocol was very useful to unambiguously rule out other explanations. Thus, overall apoB100 production may be regulated by at least two different pathways (53, 54): by post-translational degradation (23–25) and by co-translational degradation. However, mechanisms responsible for the multiple level of apoB degradation remain to be defined.

The protease involved in the co-translational degradation of apoB100 has been characterized by its sensitivity to different protease inhibitors. In a previous paper, we have shown that...
this protease is sensitive to dithiothreitol suggesting a cysteine protease (41). Ginsberg et al. (53) have also suggested that a thiol-sensitive protease could degrade apoB. Numerous proteases contains a cysteine in their catalytic sites such as the metallo-proteases, the lysosomal cathepsins, the angiotensin converting enzyme, proteases belonging to the CED3/interleukin 1β converting enzyme family, calpains, and the multicyclic protease complex called proteasome. Several of these proteases are involved in protein break down or have been suggested to regulate the level of proteins within the cell. The effect of some protease inhibitors on apoB degradation has clearly shown that the proteasome was responsible for apoB100 co-translational degradation. Such a pattern of sensitivity to these inhibitors has been observed in other processes involving the proteasome (55–57). Another characteristic of the proteasome is its ability to degrade ubiquitinated proteins (58–60).

Yeung et al. (61) have recently reported that apoB could be conjugated with ubiquitin and that MG-115 could prevent apoB post-translational degradation. Thus, we can hypothesize that apoB co-translational degradation depends on apoB co-translational ubiquitination.

All the protagonists involved in this process are not located in the same intracellular compartment. The proteasome is present in the cytoplasm or is bound to the outer side of the ER membrane but has not been detected inside the ER (62). By contrast, MTP is located in the ER lumen (1, 2). apoB is synthesized by the ribosomes on the cytoplasmic side of the ER membrane but becomes rapidly membrane-bound. apoB is translocated across the ER membrane and is assembled with lipids before being released as a free lipoprotein particle into the ER lumen (13, 19, 63, 64). The fact that the proteasome and MTP are not located in the same compartment implies that apoB co-translational degradation is not the result of a simple binding competition. MTP probably triggers the translocation of apoB100 across the ER membrane to reach the site of lipoprotein assembly. When MTP is inhibited, apoB nascent polypeptide fails to translocate across the ER membrane as it elongates. The untranslocated polypeptide probably acquires a misfolded conformation after reaching a certain length (65% of full length) thus becoming a good substrate for the proteasome. This hypothesis is consistent with the results of others that have shown that MTP is necessary for apoB translocation (34) and that untranslocated apoB is targeted for degradation (35, 65). Whether apoB translocation is co-translational (19) or post-translational (20, 21) is still a matter of controversy. Since the proteasome has not been detected in the ER lumen (62), our results imply that apoB could be exposed co-translationally on the cytoplasmic side of the ER membrane. There is no detectable accumulation of smaller proteolytic fragments in the presence of the MTP inhibitor (Fig. 3A) suggesting that almost all the apoB sequence remains accessible to cytosolic protease during the elongation. Thus, apoB translocation is not tightly coupled with translation when MTP is inhibited.

Since apoB100 synthesized in the presence of a proteasome inhibitor and the MTP inhibitor is secretion incompetent (Fig. 6), it clearly confirms that MTP activity determines the fate of apoB100 (65) and that presecretory degradation are scavenger pathways avoiding accumulation of non-functional protein. It is possible that through binding to apoB nascent polypeptide, MTP may act as a chaperone and facilitate translocation and proper folding of apoB100. The facts that MTP possess a protein disulfide isomerase subunit (66) and interacts strongly enough with apoB to be immunoprecipitated (42, 67) are consistent with this hypothesis.

Our pulse-chase experiments with synchronized polysome cells have suggested that translation of the sequences downstream the carboxyl terminus of apoB65 are more susceptible to co-translational degradation (Table I). A possible explanation is that the more apoB elongates, the longer it stays untranslated and the greater the chance for degradation. Alternatively, this could be due to a special sequence on apoB. Sequence analysis of this region of apoB100 has revealed the existence of clusters of amphipathic β-strands (the βp domain) constituting an irreversible lipid-associating domain (68). Limited trypsin digestion studies have also suggested a strong interaction of this region with core lipids on human low density lipoprotein (69). It is rather surprising that only those apoB polypeptides that have reached 65% of the full length become sensitive to co-translational degradation, since it has been shown in several experimental systems that MTP activity is required for secretion of apoB species shorter than apoB48 (5, 6). Although it is difficult to imagine that the requirement of MTP activity for apoB polypeptide elongation could be dissociated from that for apoB secretion, our data do imply that synthesis and secretion of the amino-terminal half of apoB100 may be less dependent upon MTP-mediated lipid transfer. The residual MTP activity present in cells treated with 5 µM MTP inhibitor could be sufficient to ensure translocation and lipidation of apoB nascent polypeptides smaller than apoB65. As apoB polypeptide elongates, more lipophilic β sheets are formed (68), and the requirement for MTP activity increases thus leading to misfolding on the outer side of the ER membrane and co-translational degradation. Recently, using a compound chemically similar to our MTP inhibitor, Haghpasand et al. (70) have shown that secretion of apoB48 from Caco2 cells was not decreased by this MTP inhibitor but apoB100 was. In cells lacking MTP activity, efficient translocation of apoB50 (71) and secretion of apoB41 as a lipoprotein (72) have also been reported. Furthermore, we have observed that secretion by HepG2 cells of truncated forms smaller than apoB65 was much less sensitive to the MTP inhibitor than forms larger than apoB65 including full-length apoB100. Thus, the requirement of MTP activity for both apoB polypeptide elongation and secretion depends on apoB length.

More generally, the concept that protein production could be regulated by degradation occurring during polypeptide elongation is new. Regulation of protein expression through this novel mechanism is probably related to protein folding that is initiated during translation (73). In the case of the enormous apoB100 polypeptide, it requires active MTP to translocate and to attain a properly folded conformation. The phenomenon of co-translational degradation may not be confined to apoB100 but may represent a more general mechanism regulating the production of other complex proteins. Large polypeptides fold step by step whereas the nascent polypeptide chain elongates. At some points, it is necessary for the cell to control the quality of the folding or if the polypeptide is turned into proper assembly pathway to form functional macromolecular complexes. Co-translational degradation might constitute an early check point avoiding non-functional protein from being further processed through the maturation machinery and entering the secretory pathway. It would be of interest to determine if other proteins are regulated by co-translational degradation, particularly for proteins whose production does not correlate with mRNA levels.

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REFERENCES
1. Wetterau, J. R., and Zilversmit, D. B. (1985) Chem. Phys. Lipids 38, 205–222
2. Jamil, H., Dickson, J. K., Jr., Chu, C.-H., Lago, M. W., Rinehart, J. K., Biller, 2 F. Benoist and T. Grand-Perret, unpublished results.
Co-translational Degradation of Apolipoprotein B100 by the Proteasome Is Prevented by Microsomal Triglyceride Transfer Protein: SYNCHRONIZED TRANSLATION STUDIES ON HepG2 CELLS TREATED WITH AN INHIBITOR OF MICROSOMAL TRIGLYCERIDE TRANSFER PROTEIN

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