review

Intracellular degradation of newly synthesized apolipoprotein B

Zemin Yao,[1] Khai Tran, and Roger S. McLeod

Lipoprotein and Atherosclerosis Group, Departments of Pathology and Laboratory Medicine and Biochemistry, University of Ottawa Heart Institute, 1053 Carling Avenue, Ottawa, Ontario, K1Y 4E9 Canada

Abstract Intracellular degradation of newly synthesized apolipoprotein (apo) B can occur at every stage of the secretory pathway, from the protein translation, polypeptide translocation across the membrane of endoplasmic reticulum (ER), to vesicular transport. The prevalence of apoB degradation at each stage varies in different hepatic cell systems examined. Proteolysis of nascent apoB can be catalyzed by the ubiquitin-proteasome system in the cytosol, and probably by unidentified ER resident proteases as well. Cytosolic and ER lumenal molecular chaperones that facilitate apoB translocation and folding may also assist in the degradation of misfolded apoB proteins. Factors affecting the synthesis and mobilization of lipids during lipoprotein assembly exert important regulatory effects on apoB degradation in *trans*, and specific hydrophobic amino acid sequence elements within the apoB-100 molecule may play roles in apoB degradation in *cis*. This review summarizes the current understanding of the cellular and molecular mechanisms responsible for intracellular degradation of apoB in hepatocytes. The emphasis centers primarily on the topology of apoB with respect to the ER membrane during and after apoB translation and its relationship to proteolytic mechanisms potentially involved in apoB degradation.—**Yao, Z., K. Tran, and R. S. McLeod.** Intracellular degradation of newly synthesized apolipoprotein B. *J. Lipid Res.* 1997. **38:** 1937–1953.

Supplementary key words lipoproteins • assembly • translocation • microsomal triglyceride transfer protein • proteasome • pause transfer • chaperone

INTRODUCTION

The last decade has witnessed a remarkable growth in interest in the subject of intracellular degradation of apolipoprotein (apo) B. Degradation of apoB was first observed by Borchardt and Davis (1) using primary rat hepatocytes where a significant proportion of newly synthesized apoB disappeared from the cells but was not quantitatively recovered from the culture medium. Subsequent observations from other hepatic cell models confirmed that the amount of apoB synthesized exceeds its secretion. Degradation of apoB is a rapid and efficient process. In the two most frequently used experimental systems, primary hepatocytes and hepatoma cell lines, there are differences in the extent and cellular site at which degradation of apoB predominates. In HepG2 cells, apoB degradation is confined to the early stage of lipoprotein assembly, whereas in primary hepatocytes apoB degradation occurs in both the early and late stages of secretion. This stage-dependent degradation of apoB may reflect important differences in lipid metabolism in various hepatic systems studied, particularly differences in the ability to synthesize and secrete very low density lipoproteins (VLDL) (2).

VLDL is a triacylglycerol-rich lipoprotein and one of the largest secretory macromolecules (mol wt ~20 million) of the hepatocytes. The mechanisms by which this giant lipid–protein complex is assembled and secreted are elusive, despite extensive studies. The process of VLDL assembly appears to be co-translational (3, 4); it may begin as soon as the nascent apoB polypeptide chain starts to translocate across the endoplasmic reticulum (ER) membrane. The subcellular compartment in which assembly of hepatic VLDL is completed varies among species examined. In rat hepatocytes, the VLDL particles acquire their entire lipid load within the ER (5). However, in rabbit and chicken hepatocytes, additional lipid recruitment or exchange takes place after nascent VLDL particles have exited the ER and within the Golgi apparatus (6, 7). At any point during this

Abbreviations: apo. apolipoprotein; ER, endoplasmic reticulum; VLDL, very low density lipoproteins; PMME, phosphatidylmonomethylethanolamine; SRP, signal-sequence recognition protein; TRAM, translocating chain-associated membrane protein; CHO, Chinese hamster ovary; ALLN, N-acetyl-leucyl-leucyl-norleucinal; MTP, microsomal triglyceride transfer protein; PDI, protein disulfide isomerase; HDL, high density lipoproteins; MHC, major histocompatibility complex; EST, (2S,3S)-*trans*-epoxysuccinyl-L-leucylamido-3-methylbutane ethyl ester; PI 3-K, phosphatidylinositol 3-kinase.

[1]To whom correspondence should be addressed.

lengthy maturation process from apoB translation to VLDL secretion, an inappropriately folded polypeptide or an abnormal VLDL particle can be destroyed.

The extent of apoB degradation is often inversely associated with the availability of lipids that are utilized for the assembly and secretion of VLDL (8, 9). Thus, the active synthesis, and perhaps more importantly, the mobilization of specific lipids may be the most crucial physiological regulatory factors governing the proportion of nascent apoB polypeptides that are secreted or degraded. Proper association of lipid constituents (phospholipids, triacylglycerol, and cholesteryl ester) with apoB during and after translation appears to be critical for efficient secretion of VLDL. Factors that perturb the hepatic synthesis of these lipids or impede their mobilization to the site(s) of assembly frequently block VLDL secretion and enhance apoB degradation while having very little effect on the secretion of other proteins. Thus, biosynthesis and transfer of the lipids, temporally and spatially in synchrony with apoB translation, are crucial for attainment of properly folded apoB ensuring VLDL assembly and secretion. A vital aspect of this process is the role of molecular chaperones (located in the cytosol and the lumen of the ER) in the folding and degradation of apoB.

DEGRADATION DURING TRANSLOCATION

Uncoupled apoB translocation from translation

Translocation of the 4536 amino acid apoB-100 molecule (10) into the secretory pathway represents an enormous problem for the hepatocyte. In 1990, Davis and co-workers (11) reported that translocation of apoB across the ER membrane could become arrested in rat primary hepatocytes. Exposure of a portion or the entire molecule of apoB, apoB-100, or apoB-48 (the N-terminal 48% of the full-length protein), to the cytosolic side of the ER has been detected using monoclonal antibodies with epitopes at the amino or carboxyl terminus of rat apoB-100 (12). Subsequently, incomplete translocation of apoB across the ER membrane was found in other hepatic cell models (13–18). Not only can apoB be detected on the cytosolic surface of the ER in rat liver (12), in some experiments apoB can also be detected on the cytosolic side of the Golgi fractions in chicken and rabbit livers (13, 14). In addition, the cytosolic molecular chaperone Hsp70 is found to associate with apoB in HepG2 cells (15). Under conditions where normal phospholipid composition of the ER membrane is altered by accumulation of phosphatidylmonomethylethanolamine (PMME), secretion of VLDL from rat hepatocytes is inhibited (19). Impaired VLDL secretion was a consequence of extremely inefficient translocation of apoB across the PMME-enriched ER membrane as judged by increased protease accessibility (20). Furthermore, the effect of PMME was independent of apoB polypeptide length (21). Accessibility of apoB polypeptide to exogenous proteases has also been found in isolated microsomes of rat hepatoma cells (17) or in permeabilized HepG2 cells (18). After exogenous protease digestion, several discrete bands were protected against digestion, suggesting a family of transmembrane apoB polypeptides (17, 18). Thus, while some of the apoB polypeptides may not be translocated at all, some may assume a transmembrane topology during or after translation. Although factors such as lipid availability, perturbation of lipid composition of the ER membrane, and unusual interaction between apoB and specific proteins in the translocon (see below) may dissociate translocation of apoB from translation, the apparent transmembrane topology of apoB is perplexing because classical topogenic sequences that can give rise to such a configuration do not exist within the apoB primary sequence (22–25). Davis and co-workers (11) postulated that the hydrophobic β-sheet structures within apoB might cause the protein to interact with the ER membrane. Porin, the ion-conducting channel of *Escherichia coli*, lacks a membrane-spanning domain and yet appears to exist in a transmembrane orientation. It has been suggested that hydrophobic anti-parallel β-sheets within porin may give rise to the transmembrane topology (26). Currently, a major challenge is to determine whether novel transmembrane topogenic sequences occur in the apoB molecule or whether protein components of the translocon are responsible for arresting translocation of the secretory apoB polypeptide.

Translocon and pause transfer

Only recently has the architecture of the proteinaceous channel through which secretory proteins translocate across the ER membrane (termed the translocon) begun to be elucidated (27). At a minimum, a translocon is composed of the trimeric ER membrane protein Sec61p, the heterodimeric signal-sequence recognition particle receptor (SRP receptor), and in some cases the translocating chain-associated membrane protein (TRAM) (28). The SRP receptor and TRAM are involved mainly in docking the ribosome and the initial signal sequence for translocation, whereas Sec61p defines the aqueous pore of the translocon. The aqueous channel is sealed off from the cytosolic side of the ER membrane by the ribosome, ensuring efficient co-translational translocation. Thus, during chain elongation the translocating polypeptide is usually not exposed to the cytosol. In vitro translation and translocation of a

small segment of apoB have been characterized extensively in search of candidate topogenic sequences that may induce a transmembrane configuration (29, 30). To date, putative topogenic "pause transfer sequences" have been identified within human apoB-100.

The pause transfer sequences consist of a consensus motif of LKK-T----N---A (or LKK---SE) (31, 32), present in multiple copies within human apoB-100. In vitro translation and translocation experiments show that the pause transfer sequences can engage apoB into a transient translocation stop stage and also allow subsequent restart (31). However, distinct from known topogenic sequences such as stop transfer or signal anchor sequences, whose function is autonomously demonstrable (33), the topogenic manifestation of the apoB pause transfer sequence depends strongly on the context of surrounding amino acids (31, 32). It is often observed that apoB segments containing the pause transfer sequence do not exhibit transmembrane topology (32, 34), and sometimes pausing may be caused by other abnormalities such as persistent tRNA association with peptide chains during in vitro translation (32). Therefore, whether or not the pause transfer sequence can be defined as a topogenic sequence is questionable. This unique context-dependent nature of the apoB translocation pause suggests that the pause transfer sequence may only function in the presence of certain downstream sequences whose translation may trigger restart of apoB translocation. The details of sequences mediating apoB translocation restart remain to be described.

Stalled apoB translocation may represent a regulated process. Recently a novel 11-kDa protein was cross-linked specifically to apoB segments containing the pause transfer sequence (34). It has been proposed that this 11-kDa protein is recruited into the translocon during translocation of apoB. An intriguing model proposes that interaction of the pause transfer sequence with the 11-kDa protein may interrupt the coaxial stacking of the ribosomal tunnel on the translocon pore, resulting in a tilted ribosome that allows the translating polypeptide chain to slip out into the cytosolic side of the ER membrane. Transient stop and restart in the translocation of the apoB polypeptide has been suggested to be important for lipid binding (31), but demonstration of such an effect in vivo has not been achieved. Failure to restart translocation and reassociate it with translation may lead to aborted translation or translocation, and the apoB exposed to the cytosol could become the substrate for proteasome mediated degradation (see below).

MTP and translocation arrest of apoB

The microsomal triglyceride transfer protein (MTP) is a heterodimer consisting of a large catalytic subunit (97 kDa) and protein disulfide isomerase (PDI). While PDI expression is ubiquitous, the catalytic subunit of MTP has been found to be expressed only in the liver and intestine (35), and probably in yolk sac as well (36). Transfection of recombinant apoB-53 (the N-terminal 53% of apoB-100) into Chinese hamster ovary (CHO) cells that lack the catalytic activity of MTP results in expression of an apoB polypeptide that cannot be translocated across the ER membrane or secreted (37). In CHO cells transfected with apoB-53, an 85-kDa species apparently derived from the N-terminus of apoB-53 is observed (38). A small amount of the 85-kDa species is also found in HepG2 cells where the MTP activity is present (38). Generation of this N-terminal apoB fragment appears to be catalyzed by an endogenous protease, as the protease inhibitor N-acetyl-leucyl-leucyl-norleucinal (ALLN) can effectively block its formation (38). It was thus postulated that the 85-kDa species was the product of proteolysis of a translocation arrested apoB intermediate, with the N-terminal portion translocated and the remaining C-terminal portion residing on the cytosolic surface (38). A similar species (~70-kDa) originating from the N-terminus of apoB whose abundance is reduced by ALLN (39, 40) and whose appearance is induced by exogenous proteases (18, 38) has also been observed in HepG2 cells. The 70-kDa species was also perceived as the intralumenal portion of the transmembrane form of apoB-100. Although the relationship between the 85-kDa and 70-kDa species is not entirely clear, recent experimental evidence suggests that the latter may be produced by an ER resident proteolytic mechanism (see below).

The 85-kDa species has been found in the plasma of subjects with homozygous abetalipoproteinemia (41), a disease caused by a genetic deficiency of MTP catalytic activity (42–44). This finding suggests that while translocation of full-length apoB requires MTP, small truncated apoB species can be secreted independent of MTP activity. As indicated above, transfection of human apoB-53 into CHO cells results in the expression but not secretion of apoB (37), a cellular phenotype resembling homozygous abetalipoproteinemia. Expression of a recombinant MTP catalytic subunit in non-hepatic cells transfected with apoB variants reconstitutes the assembly and secretion of lipoproteins containing apoB (45–48). MTP is therefore essential for efficient secretion of apoB-100, and may be required for apoB-100 translocation across the ER membrane (47). These in vitro results, together with observations in abetalipoproteinemia, suggest that the N-terminal apoB fragments may be derived from the translocation arrested apoB intermediates in the absence of MTP catalytic activity.

But other studies have challenged whether the presence of a protease-resistant N-terminal portion of apoB

is evidence for its transmembrane topology (49, 50). Generation of the 85-kDa species upon addition of exogenous protease was alternatively explained by resistance of this folded N-terminal domain to protease digestion (49) or by instability of microsomal vesicles (50). Resistance of folded or aggregated proteins to proteolysis, even in the presence of detergent, is not uncommon and has been reported for other proteins (51, 52). As mentioned above, in vitro translation and translocation experiments (32) and transfection studies using intact cells (53) have often failed to provide evidence for the transmembrane topology of apoB. These data cautioned against proposing transmembrane topology of a protein based solely on the presence of protease-resistant fragments. However, as mentioned above and to be discussed, the vast majority of the available experimental results indicate that translocation of apoB across the ER membrane can be uncoupled from translation. A better understanding of the potential transmembrane topology of apoB-100 awaits further identification and characterization of cellular proteolytic mechanisms that generate the 85-kDa species and other fragments derived from the amino terminus of apoB. This conflict between translocation of apoB as continuous and uninterrupted versus punctuated and delayed typifies many debates in the field of apoB research, the debate between concerted and step-wise processes for the assembly of VLDL, or between confined and extended requirement of MTP for the secretion of VLDL (see below).

Co-translational degradation

An unusual feature associated with apoB synthesis is that under certain conditions the rate of apoB translation can be decreased significantly even though the steady-state concentration of the apoB mRNA does not change (54). The discordance between apoB translation rate and apoB mRNA level suggest that either a subset of apoB mRNA is translationally inactive or that translation of some apoB polypeptides never reaches completion. Direct measurement of the rate of hepatic apoB translation has been performed using the liver of streptozotocin-induced diabetic rats (54). The forms of apoB synthesized by hepatocytes derived from the diabetic rats were predominantly apoB-48, and the rate of apoB translation was 4-times slower than in hepatocytes derived from non-diabetic animals, despite unchanged levels of the apoB mRNA (54). Thus in the diabetic condition, elongation of the apoB polypeptide chain is impaired and may terminate near the C-terminus of apoB-48. Decreased apoB translation was also observed in insulin-treated rat hepatocytes and in insulin-treated HepG2 cells, as determined by incorporation of radiolabeled methionine (55) or by in vitro translation using cell extracts (56) where the apoB mRNA level was unchanged. Unfortunately, the latter studies did not determine whether truncated apoB species were produced in the insulin-treated cells. Although inconclusive, the available evidence favors a model of co-translational degradation of apoB in which proteolysis of the apoB polypeptide can occur during chain elongation. In HepG2 cells, degradation of the elongating apoB polypeptide chains could be blocked by ALLN (57), suggesting proteolysis, probably catalyzed by proteasomes, may occur at the stage of protein translation (see below). Unlike post-translational degradation where the entire apoB-100 polypeptide is destroyed, co-translational degradation usually produces an N-terminal portion of apoB that can be secreted, sometimes as part of a lipoprotein. Models for co-translational degradation of apoB are shown in **Fig. 1.**

Difference between apoB-100 and apoB-48 in translocation

Although the physiological significance of having two forms of apoB, apoB-100 and apoB-48, in mammals remains unclear (58), differences in their metabolism were observed some time ago (59–62). Recently, differences between apoB-100 and apoB-48 have also been observed in the requirement for MTP activity during translocation. In transfected non-hepatic cells expressing short apoB variants (i.e. $\leq$ apoB-48) or when assembly of apoB-48-containing lipoproteins is reconstituted in vitro, MTP activity appears not to be required for apoB translocation, even though formation of lipoproteins containing these apoBs requires MTP activity (47, 63). Co-expression of MTP in non-hepatic cells or inactivation of MTP in hepatic cells did not alter the sensitivity of the short apoBs to exogenous proteases (64). These results, together with the observation that apoB-41 can be secreted as particles resembling high density lipoproteins (HDL) from a transfected murine mammary-derived cell line that lacks MTP (65), indicate that the short apoBs require relatively little or no MTP activity for translocation or secretion.

Translocation of apoB-100 and other large truncated forms of apoB (i.e. $\geq$ apoB-60), in contrast to apoB-48, is influenced by alterations in MTP activity. A 220-kDa species, termed B-48-like protein, is produced and secreted by COS-7 and McA-RH7777 cells overexpressing large human apoB variants (i.e. $\geq$ apoB-60) (47, 66, 67). Formation of this 220-kDa species is not the result of degradation of full-length apoB-100 after translation; pulse-chase experiments revealed that production of the B-48-like protein is co-translational and independent of apoB mRNA editing (47, 68). Unlike authentic apoB-48 that is inaccessible to exogenous proteases in isolated microsomes and can form VLDL in McA-

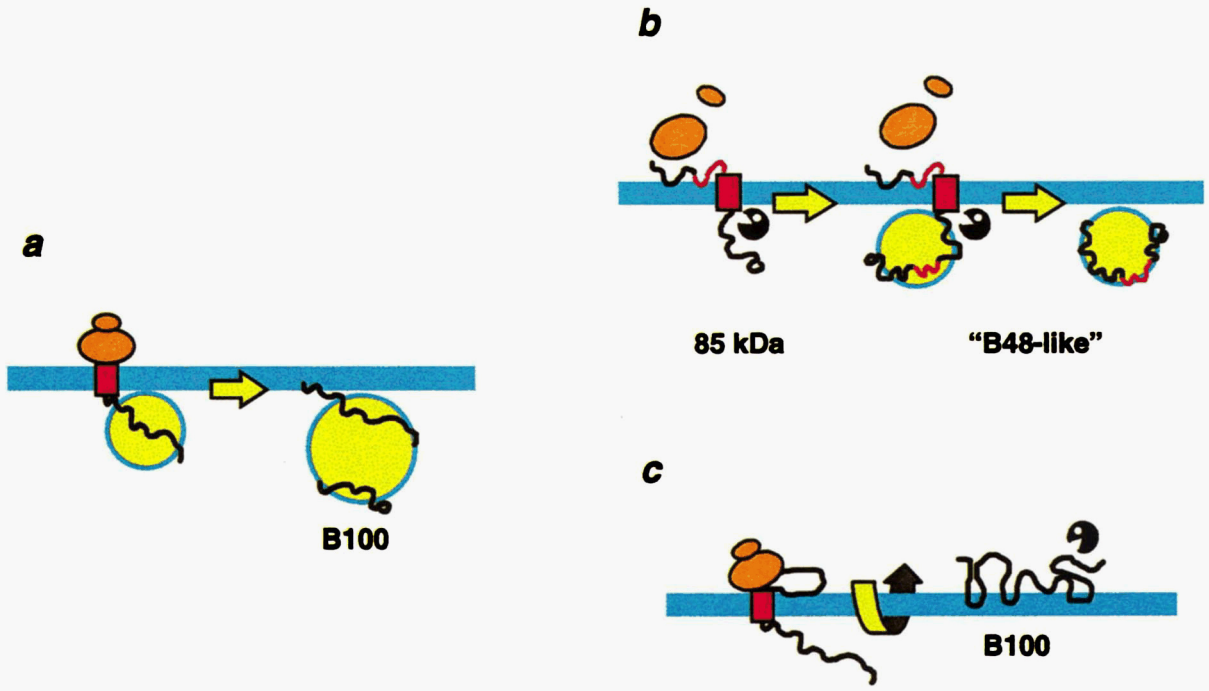

Fig. 1. Models of co-translational degradation of apoB. *a*, Co-translational assembly occurs during translocation through the translocon (shown as a red rectangle). In HepG2 cells, products of the co-translational assembly are mainly the triacylglycerol-rich LDL that are secreted as such, whereas in primary hepatocytes and McA-RH7777 cells they acquire additional lipid to form VLDL. *b*, Aborted translation and proteolytic cleavage of the translating apoB polypeptides resulted from arrested translocation. In HepG2 cells, translocation arrest and cleavage may occur at the N-terminus of apoB-100 yielding the 85-kDa species (38), whereas in COS-7 and McA-RH7777 cells they may happen at the middle of the apoB-100 polypeptide yielding B-48-like protein (47). Translocation arrest may be mediated by the putative *cis* elements (red segments) within apoB molecule. It is unknown whether proteolytic cleavage occurs on the cytosolic (not shown) or on the lumenal side (as shown) of the ER. *c*, Delayed translocation causes ribosome to tilt and allows nascent apoB polypeptide to escape the translocon into the cytosolic side of the ER membrane. Unassembled or misfolded apoB-100 polypeptides (partly or fully translated) may be dislocated into the cytosol (indicated by an inverse arrow) and degraded by the cytosolic proteasome (78).

RH7777 cells upon oleate supplementation, the B-48-like proteins are sensitive to proteolysis and unable to assemble VLDL (R. S. McLeod and Z. Yao, unpublished results). In McA-RH7777 cells transfected with human apoB-100, a 7-kilobase apoB mRNA predominates and associates strongly with the formation of B-48-like proteins (69). It was postulated that activation of cryptic polyadenylation sites in the middle of the human apoB-100 mRNA might generate the shortened apoB message and yield the B-48-like proteins (69, 70). However, using cells in which the MTP activity can be manipulated, we have obtained evidence suggesting that B-48-like proteins may represent the products of co-translational cleavage of apoB. Coexpression of the MTP catalytic subunit with apoB in COS-7 cells reduced the formation of B-48-like species and reciprocally increased the full-length apoB without changing the level of the 7-kilobase apoB RNA (47). In addition, accumulation of the apoB intermediates with molecular mass of 100- to 200-kDa has been observed in HepG2 cells in which MTP activity is inhibited (71). This was accompanied by impaired conversion to intermediates larger than 320-kDa and was due to proteolysis that could be inhibited by the proteasome inhibitor lactacystin, rather than aborted chain elongation (71). These results, when combined, support the hypothesis that formation of B-48-like proteins results from co-translational degradation of apoB transmembrane intermediates (47). As formation of B-48-like protein was found only during translation of large apoB forms (≥ apoB-60), we have postulated that sequences within the C-terminal half of apoB-100 may act in *cis* causing co-translational degradation. The *cis* elements, probably residing within the domain enriched with amphipathic β-strands (72), could be responsible for translocation arrest of apoB-100 (Fig. 1b). However, the relevance of these cell culture results to the in vivo situation is unclear as the B-48-like proteins are not found in the plasma of homozygous human abetalipoproteinemia (41). What remains to be obtained is definitive in vivo experimental evidence that the putative *cis* elements indeed exist within apoB-100 that can cause interrupted transloca-

tion across the ER membrane resulting in secretion of B-48-like proteins. Transgenic animals that express an inactive MTP catalytic subunit will be useful in vivo tool to verify and extend these cell culture observations.

Degradation on the ER membrane

Unlike typical secretory proteins that are translocated to the ER lumen after translation, significant proportions of newly synthesized apoB polypeptides have been found to associate with the ER membrane. In rat hepatocytes, the membrane-associated apoB is accessible to exogenous protease whereas the lumenal apoB is insensitive (11), indicating some apoB polypeptides are located on the cytosolic surface of the ER. Data obtained from pulse-chase experiments suggest that the newly synthesized, protease-accessible apoB is not the precursor of lumenal apoB and is retained in the ER (11). Conversely, kinetic studies show that the membrane-associated apoB is not derived from the lumenal species after translocation (73). It has been reported that in intact (74) or in permeabilized (75) HepG2 cells, some membrane-associated apoB may serve as the precursor of the lumenal apoB associated with particles of high density. However, unlike the co-translationally assembled lumenal apoB-containing LDL–VLDL that are secretion competent, these HDL-like apoB-containing particles are not secreted from the cells nor are they converted into secretion-competent species (73, 75). Thus, the membrane-associated apoB proteins most likely represent a non-translocated or partially translocated pool of unassembled apoB that will be degraded on the ER membrane.

Until recently, degradation of unassembled or aberrant apoB retained in the ER was thought to involve only ER-localized proteases, either on the lumenal or on the cytosolic side (76, 77). It is now recognized that some of the apoB-100 polypeptides are degraded in the cytosol by the proteasome. In HepG2 cells, degradation of apoB-100 could be blocked by the proteasome inhibitor MG115 or by ALLN. Concomitantly, ubiquitinated apoB, presumably the substrate for the proteasome-mediated degradation, accumulated within the cells (78). Degradation of apoB-100 was also inhibited by depletion of ATP with dinitrophenol and 2-deoxyglucose, further implicating the ATP-dependent, ubiquitin-proteasome pathway in apoB-100 degradation.

Proteasomes are large multi-subunit protease complexes that play a central role in nonlysosomal protein degradation (79). Like apoB-100, most of the proteins degraded by these proteases are tagged by ubiquitination, involving the attachment of multiple chains of ubiquitin, a protein consisting of 76 amino acids. Ubiquitinated proteins are marked for ATP-dependent hydrolysis by a 26S complex, composed of a 20S multicatalytic protease core and two 19S cap complexes thought to be required for the recognition of ubiquitinated proteins (80). However, ubiquitination is not always a prerequisite for the proteasome-mediated protein degradation. For example, the transmembrane major histocompatibility complex (MHC) class I molecules (81) and secretory αl-antitrypsin Z (82, 83) are not ubiquitinated but are degraded by the proteasome. For degradation of ER membrane proteins or secretory proteins by the proteasome, the entire polypeptide chains must traverse the ER bilayer back into the cytosol. It has been recently shown that the heavy chain of MHC class I (a type I membrane protein) can disintegrate from the ER membrane and move into the cytosol via a reversal of translocation, a process termed dislocation. Interestingly, the same translocation channel, the Sec61 translocon, is apparently used for protein dislocation (81). To date, factors mediating this reverse translocation of membrane proteins into the cytosol have not been fully characterized. Topologically, nascent apoB polypeptides that are associated with the ER membrane could potentially serve as candidate substrates for the proteasome degradation. It is tempting to speculate that the incompletely translocated apoB molecules, as observed in intact and permeabilized HepG2 cells (75), may undergo dislocation into the cytosol for destruction (Fig. 1c), analogous to the heavy chain of MHC class I molecules. The observation of proteasome-mediated apoB-100 degradation in HepG2 cells (78) is in good agreement with the previously reported non-lysosomal, pre-Golgi, ATP-dependent, and ALLN-sensitive characteristics associated with intracellular degradation of apoB (39, 76, 77, 84). The proteasome-mediated degradation may also be responsible for the observations in CHO cells expressing recombinant apoB, in which apoB degradation could be inhibited by ALLN (37). Protein or lipid factors that regulate the gating of the translocon or "dislocon" for the movement of apoB polypeptides across the ER membrane remain to be identified.

Degradation of proteins by the ubiquitin-proteasome pathway may be assisted by molecular chaperones. Although generally considered to play a role in protein folding and translocation, molecular chaperones can also play a role in facilitating degradation of abnormal proteins (85). Interaction of Hsp70 with apoB-100 has been observed in HepG2 cells, and this interaction intensifies when the cells are cultured in the absence of lipid substrate (15). Interestingly, inhibition of the cytosolic proteasome, using ALLN or lactacystin, induced Hsp70 gene expression by stabilizing the *hsp70* transcription factor HSF1 (86). In addition, induction of endogenous Hsp70 using an ansamycin antibiotic or by overexpressing recombinant Hsp70 in HepG2 cells en-

hanced the proteasome-mediated degradation of apoB (87; E. Fisher and H. N. Ginsberg, personal communication). Thus, some of the inhibitory effect of ALLN on apoB degradation described above may be offset by the ALLN-induced Hsp70 expression. It has recently been reported that the composition of serum-free culture medium also profoundly influences the spontaneous induction of Hsp70 in cultured rat hepatocytes (88). Therefore, in future experiments it should be considered that environmental stimuli may potentially affect apoB degradation via altered expression of Hsp70 or other cytosolic chaperones. Binding of Hsp70 to apoB-100 may either facilitate apoB-100 translocation across the ER membrane or stimulate the breakdown of apoB-100. This "refold or degrade" model (85) for Hsp70 remains to be tested experimentally. The discovery of degradation of newly synthesized apoB by proteasomes and the observation of interaction between Hsp70 and apoB-100 strongly support the model that some apoB polypeptides are exposed to the cytosolic side of the ER membrane during and after translation.

Degradation signal?

Signals triggering intracellular degradation of apoB by the ubiquitin-proteasome pathway have not been identified. The *cis* elements within apoB-100 such as the PEST motif (sequences enriched with proline, glutamic acid, serine, and threonine (89)) or sequences homologous to those in targeting the T cell receptor-α for ER degradation have been postulated to play a role in apoB degradation (11, 90). In addition, post-translational modification of apoB such as phosphorylation (91) has also been suggested to be involved in degradation of newly synthesized apoB. However, experimental evidence demonstrating their function as a degradation signal is lacking.

Human apoB-100 is a glycoprotein having 19 potential N-glycosylation sites of which 16 are found to be utilized in the plasma low density lipoproteins (92). The glycosylated apoB-100 has been shown to bind to calnexin in HepG2 cells (93) and in transfected COS cells (48). Calnexin is an ER resident chaperone protein that binds preferentially to glycoproteins containing the trimmed Glc_1–Man_9–$GlcNac_2$ oligosaccharide moiety and participates in the quality control process involving glucosylation and deglucosylation of N-linked glycoproteins (94). Several experiments have suggested that calnexin may play a role in the proteasome-mediated degradation of mutant or unglycosylated secretory proteins such as α1-antitrypsin Z (52, 83). It is unclear whether calnexin also plays a role in the quality control system for apoB. Inhibition of N-glycosylation of apoB by tunicamycin abolishes calnexin binding to apoB but has no detectable effect on apoB secretion from chicken hepatocytes (95) or from transfected COS cells (48). Thus, factors other than calnexin may be more important in signaling proteasome-mediated apoB degradation. However, differences in the extent of glycosylation have been observed between the apoB species that are associated with rat hepatocyte membranes and apoB in the plasma; the former is more extensively glycosylated than the latter (96). Details of deglycosylation during the assembly and secretion of apoB-containing lipoproteins or in plasma have not been described.

Prevailing evidence suggests that insufficient supply of specific lipids during lipoprotein assembly is the major *trans* factor signaling intracellular degradation of apoB. In transfected cell lines, the stability of apoB appears to be inversely related to its length or lipid-binding ability. The N-terminal 17% of apoB has limited ability to bind lipids (97), and it does not exhibit significant intracellular degradation in transfected cells despite low secretion efficiency (66) (**Fig. 2**). As the length of apoB increases (i.e., ≥ apoB-29), the secretion efficiency and the stability of most apoBs examined decline, indicative of increased intracellular degradation. The size of apoB-lipoprotein products of co-translational assembly (i.e. the HDL or LDL particles) is a function of apoB length (3, 4, 66). Likewise, the dependence on MTP activity for secretion of these products increases with the apoB length as well (47). Thus, the demand for adequate lipid association for apoB to attain correct conformation is a function of apoB length. With few exceptions, the results of apoB stability and secretion efficiency obtained from transfected cells are in agreement with in vivo observations of naturally occurring apoB mutants found in human hypobetalipoproteinemia. The short apoB forms such as apoB-25 (98) and B-27.6 (99) were undetectable in the plasma of hypobetalipoproteinemia. Likewise, recombinant apoB-18 and B-23 were poorly secreted from transfected McA-RH7777 cells (66). The production rates of large apoB forms such as apoB-75 and B-89 in vivo were comparable to those of apoB-100 in heterozygous hypobetalipoproteinemia (100). Recombinant apoB-72, B-80, and B-100 also had similar secretion efficiencies in transfected cells (67). Discrepancy between in vivo and cell culture studies lies in the secretion of apoB forms with intermediate size (i.e. apoB-31 to B-54.8). In heterozygous hypobetalipoproteinemia, the in vivo production rate of truncated apoBs was directly related to apoB length (100), inferring that secretion of apoB-54.8 was more efficient than that of apoB-31. In cell culture studies, on the contrary, secretion efficiency (i.e., the proportion of total apoB that is secreted) of truncated apoBs (between apoB-29 and B-60) is inversely related to apoB length (Fig. 2). Thus, the in vitro data suggest that the low plasma level of apoB in hypo-

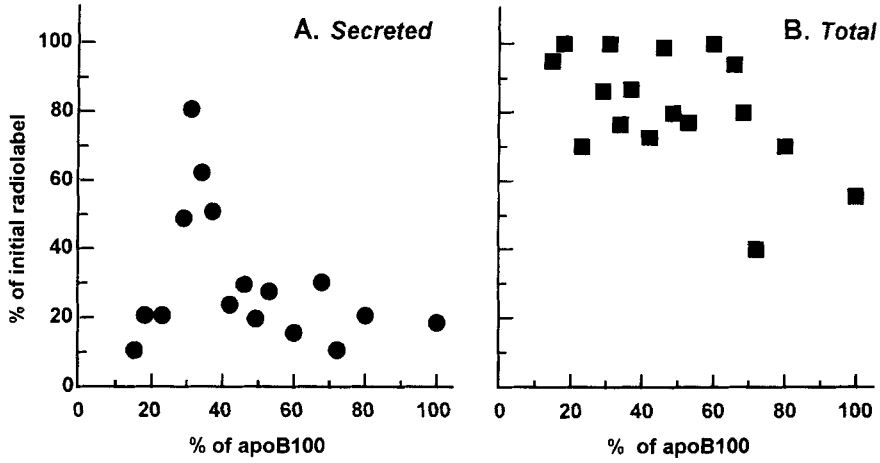

Fig. 2. Panel A, secretion efficiency of truncated forms of human apoB determined by pulse-chase experiments using stably transfected McA-RH7777 cells. Cells were pulse-labeled with radiolabeled amino acids for 2 h in a serum-free medium, and radioactivity associated with cell and medium apoBs were quantified at the beginning and the end of 2-h chase. Secretion efficiency is presented as the percent of total radiolabeled apoB that is secreted into the medium. Panel B, stability of truncated forms of human apoB in stably transfected McA-RH7777 cells. Stability of apoB is presented as the percent of total apoB that can be recovered (medium + cell) at the end of chase. Data are compilation of published results (21, 66, 67, 102–105) except for apoB-34, B-42, B-46, and B-64 (R. McLeod, S. Selby and Z. Yao, unpublished results).

betalipoproteinemia is not attributable to impaired secretion. The low steady state level of truncated apoBs, such as apoB-31, observed in vivo may also be explained by increased catabolism in the plasma (101). An alternative explanation, however, is enhanced post-translational degradation prior to secretion. Factors responsible for the in vivo post-translational degradation of apoB-31 or other apoB forms that exhibit efficient secretion in cultured cells remain to be determined.

DEGRADATION AFTER TRANSLOCATION

To date, mechanisms by which apoB is degraded after complete translocation have not been fully defined. While it is relatively easy to envision that an incompletely translocated apoB might be accessible on the cytosolic side of the ER membrane for proteasome-mediated degradation, it is rather difficult to perceive such a degradation mechanism for a fully translocated lumenal apoB that is associated with an assembled lipoprotein particle. Therefore, novel degradation processes might exist within the ER, or in distal compartments of the secretory pathway, whereby the misfolded or aberrant apoB-lipoproteins could be removed.

Degradation within the ER

In HepG2 cells, apoB-100 molecules associated with HDL-like particles have often been observed in the lumen of microsomes (3, 74). As discussed above, these apoB-100–HDL particles might originate from apoB-100 polypeptides that initially associated with the microsomal membranes (74, 75). Under no circumstances, however, were these apoB-100–HDL particles found in the culture medium of the cells, suggesting that the poorly lipidated apoB-100 is recognized by a quality control mechanism and subsequently degraded within the ER.

Some ER resident proteins, such as immunoglobulin heavy chain binding protein (BiP/GRP78), PDI, and calnexin, assist in the folding of polypeptides at the beginning of their synthesis. These proteins, together with ER resident proteases, constitute a quality control system to help ensure that polypeptides are correctly folded to exit the ER and that incorrectly folded polypeptides are eliminated. Multiple ER proteases are probably involved. Protease ER-60 has been characterized as a cysteine protease in the ER of rat liver by virtue of its sensitivity to the cysteine protease inhibitor ALLN (106). ER-60 is regarded as a member of the PDI family and contains two copies of the internal thioredoxin motif, CGHC. Studies of degradation of misfolded mutant human lysozyme in mouse L cells have suggested that ER-60 and PDI may act in a concerted manner to assist protein folding and to mediate protein degradation in the ER (107).

Another ER resident protease, ER-70, has been characterized from rat and mouse liver (108). The sensitivity of ER-70 to protease inhibitors was the same as that of ER-60. In addition, the proteolytic activities of both ER-70 and ER-60 were inhibited by acidic phospholipids

such as phosphatidylinositol and phosphatidylinositol 4,5-bisphosphate (108). In HepG2 cells, ER-60 and PDI can be cross-linked to apoB-100, raising the possibility that ER-60 may play a role in apoB degradation and in generation of the 70-kDa fragment within the ER lumen (109; K. Adeli, personal communication). The functional role of the isomerase activity of PDI in assisting apoB secretion has also been demonstrated in an insect cell line expressing human apoBs (110). Interestingly, when acting as a co-factor for MTP, the isomerase activity of PDI was not required for apoB secretion from the insect cells (110). In addition to Hsp70, interaction of apoB-100 with MTP (111), calnexin (93), and BiP (48) has also been observed in HepG2 cells. Thus, an interplay between multiple molecular chaperones and the ER proteases in the control of apoB folding and apoB degradation has begun to be unraveled.

Misfolded lumenal proteins can be transported back to the cytosol for proteasome-mediated degradation, a process termed retrograde translocation, as described in yeast and in vitro systems (112, 113). Should the same mechanism operate in mammalian cells, could a misfolded, fully translocated lumenal protein such as apoB-100 become a substrate for the cytosolic proteasome? Early studies with HepG2 cells (74) showed that nascent apoB-100 polypeptides, in the ER lumen or membrane-bound, were glycosylated (labeled by [^{3}H]mannose), suggesting that the glycosylated apoB-100 in HepG2 cells may be the substrate for degradation in the ER. Another experimental evidence for potential retrograde translocation of apoB came from in vitro translation and translocation of the C-terminal 20% of human apoB-100. In the presence of microsomal membranes, the expressed apoB segment was fully accessible to exogenous proteases even though it was glycosylated (50). In addition, chimeric proteins containing a segment of apoB that were expressed in McA-RH7777 cells were translocated (as judged by resistance to exogenous proteases) but their degradation was sensitive to ALLN (114). These observations raise the possibility that glycosylated apoB polypeptides, translocated fully or in part, may become exposed to the cytosol for degradation by the proteasome, in addition to degradation by proteases within the ER lumen. Recombinant apoBs that are expressed either in cell culture or by in vitro translation/translocation should be used to test this possibility.

Degradation in post-ER compartments

Notable differences have been recognized in the intracellular degradation of apoB among various hepatoma cell lines and primary hepatocytes. In most primary hepatocytes examined, degradation of apoB is not confined to the ER (115) and degradation may occur even after an apoB-lipoprotein is formed (16, 116–119). Combined experimental results indicate that degradation of apoB can occur in all secretory compartments by a variety of unidentified proteases, and the mode of degradation may vary even from one cell preparation to another.

In rat primary hepatocytes, brefeldin A plus nocodazole completely blocked apoB degradation, whereas monensin and lysosomotropic agents such as ammonium chloride and chloroquine partially inhibited apoB degradation (16). These data, in sharp contrast to the non-lysosomal and ER degradation observed in HepG2 cells (39, 76, 77), suggest that apoB degradation in rat primary hepatocytes occurs in a post-ER compartment or in lysosomes. In an in vitro assay, incubation of purified Golgi fractions of rat hepatocytes at 40°C resulted in apoB degradation while little apoB was degraded in purified ER fractions (16), suggesting that there might be a Golgi resident protease responsible for apoB degradation. In contrast, similar experiments performed using HepG2 cells showed that apoB-100 was degraded by an ER-resident protease and that there was no apoB degradation in the Golgi (77). The Golgi-degradation of apoB can be inhibited by a cysteine protease inhibitor EST, or (2S,3S)-*trans*-epoxysuccinyl-L-leucylamido-3-methylbutane ethyl ester in rat hepatocytes (16). For reasons that are not clear, the widely used proteasome inhibitor ALLN (37, 120) was found to be either unable to reproducibly inhibit apoB degradation (16, 121) or cytotoxic (118) in rat primary hepatocytes. These results have exemplified that the predominant modes of apoB degradation may vary even among rat hepatocyte preparations.

In chicken primary hepatocytes, degradation of apoB appears to occur in both the ER and the Golgi compartment. Using inhibitors of metalloproteases, serine proteases, serine/cysteine proteases, or cysteine proteases, Cartwright and Higgins (119) have found that different proteases may be responsible for apoB degradation at different compartments. While *o*-phenanthroline (a metalloprotease inhibitor) blocked apoB degradation in the rough ER and smooth ER, leupeptin (a serine/cysteine protease inhibitor) blocked apoB degradation in the smooth ER and *cis*-Golgi (119). Although the effect of leupeptin on apoB-100 degradation was not observed in HepG2 cells (76), in rat primary hepatocytes it also inhibited apoB-100 degradation by 20–30% (16). Interestingly, ALLN inhibited apoB-100 degradation in the *trans*-Golgi in chicken primary hepatocytes, and the accumulated apoB-100 polypeptides were associated with the cytosolic side of the *trans*-Golgi membrane and were diverted from secretion (119). The ALLN-sensitive, post-Golgi degradation of other VLDL apolipoproteins (e.g., apoE) was also observed in HepG2 cells

(122, 123). The cytosolic exposure of apoB-100 and its degradation by an ALLN-sensitive protease in the Golgi compartment may suggest that proteasome-mediated degradation of apoB also occurs in post-ER compartments.

Degradation after lipoprotein assembly

Evidence of apoB degradation after lipoprotein assembly was obtained from studies with choline-deficient rat hepatocytes (105, 116, 118) and n–3 fatty acid-treated hepatic cells (117, 124, 125). Defects in phosphatidylcholine synthesis led to formation of VLDL particles with altered phospholipid composition (i.e., increased phosphatidylethanolamine and decreased phosphatidylcholine) and stimulated degradation of these particles. Provision of n–3 fatty acids (eicosapentaenoic acid and docosahexaenoic acid derived from fish oil) also enhanced intracellular degradation of VLDL in post-ER compartments, presumably as a consequence of altered lipid composition. The stimulation in degradation was more pronounced for particles of low buoyant density (i.e., more lipidated) than for the less lipidated apoB (105, 125), suggesting that the abnormal lipid composition may play an important role in targeting apoB to the degradation mechanism.

It is not clear why VLDL particles with altered lipid composition would be "sensed" as abnormal and be destroyed by the quality control system. Considering the high degree of heterogeneity in the size of secreted hepatic VLDL (126, 127) that undoubtedly have different lipid composition and will inevitably affect apoB conformation, one might predict that composition of lipids should not be the major factor in determining intracellular apoB degradation. Indeed, significant amounts of VLDL with altered phospholipid composition are secreted from the choline-deficient hepatocytes (116, 128). Another compelling piece of evidence that degradation is not triggered by the lipid composition of a lipoprotein comes from secretion of apoB-48 from rat hepatocytes. Rat hepatocytes synthesize and secrete both apoB-100 and apoB-48, and while apoB-100 is secreted predominantly as VLDL, apoB-48 is secreted as either VLDL or HDL (129). It is now clear that B-48–HDL is the product of co-translational assembly and can be secreted as such if no further lipid recruitment occurs. Alternatively, some of the B-48–HDL particles may undergo a second lipidation stage, expanding the lipid content to form B-48–VLDL (130, 131). As discussed below, under many pathophysiological conditions where B-48–VLDL secretion is impaired, hepatic secretion of B-48–HDL is not affected. Obviously, the secretory pathway for VLDL is more sensitive to degradation than that for HDL. However, the ability to secrete either B-48–HDL or B-48–VLDL indicates that both lipoprotein species are "viewed" by the cells as normal. Therefore, degradation of apoB-48, either as HDL or as VLDL, is unlikely determined solely by the composition of lipoprotein lipids. Rather, factors that modulate the vesicular transport system for VLDL assembly and secretion may play a more important role in the degradation process. What has not been excluded in the above choline-deficiency or n–3 fatty acid studies is the possibility that stimulated VLDL degradation is caused by disruption of a putative vesicular transport system specific for apoB that could be sensitive to the perturbed phospholipid synthesis or by accumulation of abnormal lipids.

Degradation during vesicular transport

To date, the existence of a specialized vesicular transport system specific for apoB-containing lipoprotein secretion has not been described. Considerable experimental evidence suggests that the secretory pathway for apoB may not be simply the classic ER-to-Golgi route responsible for the export of bulk secretory proteins. It has been found that polysomes that contain apoB mRNA exhibit unusual physical properties (132) and localize to a specialized intracellular compartment (termed low density membranes) in rat hepatocytes (133). Thus, translation and co-translational degradation of apoB may occur in an atypical ER environment. Additionally, while secretion of bulk proteins (including apoB-48–HDL) was normal, secretion of VLDL (both apoB-100–VLDL and apoB-48–VLDL) could be significantly decreased under many pathophysiological or pharmacological conditions, including inhibition of phosphatidylcholine synthesis by choline deficiency (128), inactivation of MTP catalytic activity by inhibitors (114, 134), inhibition of phosphatidylethanolamine methylation by bezafibrate (135), treatment with orotic acid (73, 136, 137), n–3 fatty acids (117), insulin (54), dexamethasone (138), or monomethylethanolamine (21). Although some of the inhibition of VLDL secretion was caused by impaired apoB translocation across the ER membrane at the early stage of VLDL assembly (21), the others may result from an altered vesicular transport system leading to post-ER degradation of apoB.

An intriguing observation was made in the rat hepatoma McA-RH7777 cells that hepatic apoB-48–VLDL assembly and secretion was extremely sensitive to brefeldin A (134, 139). Brefeldin A is a fungal metabolite that blocks the binding of coatomer and ADP ribosylation factors (a family of low molecular weight GTP binding proteins) to the membrane and thus the budding of coated vesicles (140, 141). At concentrations that permitted normal secretion of bulk hepatic secretory proteins including apoB-48–HDL, brefeldin A abolished formation and secretion of apoB-48–VLDL (134, 139). In McA-RH7777 cells, synthesis and secretion of B-48–VLDL have been shown to be achieved through a "sec-

ond step," a process that can be induced by exogenous oleate (17, 130, 134). Although the nature of this second lipid assembly step has not been fully characterized, it appears to be sensitive to inhibition of protein synthesis (130) in addition to the brefeldin A-sensitive elements (134, 139). The second-step lipid assembly may also require the activity of MTP, although to date results concerning the requirement of MTP are inconsistent. Working with McA-RH7777 cells treated with an MTP inhibitor, Gordon and co-workers (142) found that inactivation of MTP had no effect on the oleate-induced assembly and secretion of apoB-48–VLDL whereas the secretion of apoB-48–HDL, the product of first-step assembly, was inhibited. These results suggested that MTP might not play a role in the recruitment of bulk core lipid in apoB-48–VLDL assembly. Using McA-RH7777 cells transfected with human apoB variants, we have found that the requirement of MTP activity for apoB secretion is inversely related to apoB length (114). Moreover, we have found that normal activity of MTP is required for the oleate-induced secretion of human apoB-48–VLDL by transfected McA-RH7777 cells (134). At low doses of MTP inhibitor where MTP activity was reduced by 65–70%, secretion of apoB-48–HDL was normal but incorporation of triacylglycerol into apoB-48–VLDL was severely compromised (134). We have postulated that MTP may play a role both in facilitating formation of ER-associated triacylglycerol droplets *at* the site and in transport of lipid *to* the site of the second step, processes that could also be sensitive to brefeldin A (134). Further experiments using primary hepatocytes or animals will help to ascertain the functional role of MTP in hepatic VLDL assembly and secretion. In addition, the relevance of the "two-step" assembly process for apoB-48–VLDL to the physiology of human apoB-100–VLDL also needs to be established.

A more intriguing observation was made in rat hepatocytes that insulin-stimulated apoB degradation required an intracellular trafficking event and phosphatidylinositol 3-kinase (PI 3-K) activation (121). Inactivation of PI 3-K by the inhibitor wortmannin abolished insulin-dependent inhibition of apoB secretion, and protease inhibitors EST and ALLN blocked the insulin-stimulated apoB degradation. These observations are the first demonstration that the signaling events elicited by insulin can extend to the secretory compartment involved in VLDL assembly and secretion. A well-described example of vesicular trafficking mediated by activation of PI 3-K by insulin receptor substrate-1 is the rapid translocation of GLUT-4, a glucose transporter, from the low density membranes to the plasma membrane in adipocytes (143). Thus, a specialized vesicular transport system may exist for the specifically regulated synthesis and degradation of hepatic apoB.

In rat hepatocytes, the effect of insulin on apoB synthesis and secretion is antagonized by dexamethasone (138). Degradation of apoB could be blocked by protease inhibitor EST in the Golgi fractions and was also inhibited by dexamethasone in intact hepatocytes (16). Thus, the opposing effects of insulin and dexamethasone may regulate hepatic apoB synthesis and secretion in a common compartment. Immunohistochemical examination of hepatocytes treated with EST showed that the accumulated apoB did not colocalize exactly with α-mannosidase II, although both occurred in the Golgi region of the cell (138). These findings provide new evidence that a sorting mechanism may exist in the Golgi compartment that regulates post-translational degradation and secretion of apoB-containing lipoproteins in primary hepatocytes. Our current proposal of post-translational degradation of apoB is summarized in **Fig. 3.**

CONCLUDING REMARKS AND FUTURE DIRECTIONS

Protein degradation is an important mechanism of cellular regulation. Regulation of hepatic apoB levels by intracellular degradation may offer some advantages over other control mechanisms by enabling the cells to rapidly adjust to the need to secrete triacylglycerol into the circulation without invoking the translation of this enormous protein. However, several questions remain to be addressed. Does the intracellular degradation of apoB indeed reflect a regulated process that actively governs the output of hepatic VLDL? Or is it merely an unremarkable waste disposal process representing a consequence of aborted assembly or failed secretion? A general answer to these questions may not be easily obtained, as mechanisms responsible for apoB degradation may operate distinctly in different hepatic cell models. Thus, while the fate of newly synthesized apoB-100 in HepG2 cells can be primarily determined by regulated apoB translocation across the ER membrane (57), similar conclusions may not be applicable to primary hepatocytes because in these cells both ER and post-ER degradation occurs. In addition, the extensive apoB degradation in HepG2 cells may be exaggerated by the inability of the cells to mobilize lipid for VLDL assembly. Moreover, the lack of an effect of exogenous oleate on apoB secretion and degradation in primary hepatocytes also challenges the physiological relevance of the lipid-mediated apoB production in hepatoma cell lines. In vivo investigations using transgenic animals with targeted inactivation of molecular chaperones such as Hsp70 and MTP may help to verify the apoB degradation mechanisms demonstrated in cell model systems. Finally, as most of the data concerning the loca-

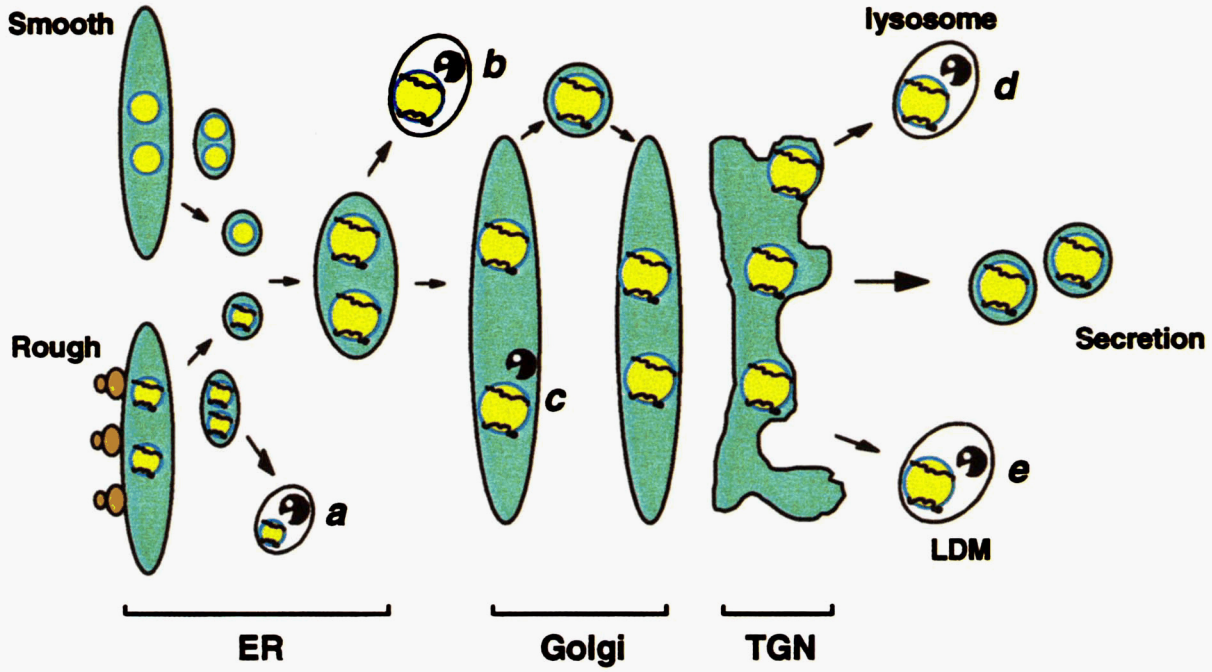

Fig. 3. Degradation of apoB after assembly. *a*, Degradation of apoB within the ER or ER-derived vesicles due to insufficient supply of lipid at site(s) for the second-step assembly (74, 75). Mobilization of lipids from the smooth ER to the assembly sites may involve ER vesicularization or other membrane trafficking events that are sensitive to brefeldin A or MTP inhibition. *b*, Post-ER, pre-Golgi degradation of apoB associated with aberrant lipoproteins (116–119). *c*, Degradation of apoB within the Golgi apparatus (119). *d*, Degradation of apoB in lysosome (16). *e*, Degradation of apoB induced by insulin in a specialized membrane fraction (i.e., low density membranes) (121, 131) and may be antagonized by dexamethasone (16, 138).

tion and mechanisms of apoB degradation were generated using inhibitors of proteases or organelle function, caution must be exercised in interpreting the data because some of the inhibitors may not be specific and may exert unknown cellular effects.

Regardless of the differences in post-translational degradation of apoB in various primary hepatocytes or hepatoma cell lines, similarities do exist. In the last decade, identification of protein factors, such as MTP, essential for VLDL assembly and recognition of the ubiquitin–proteasome pathway for ER degradation of apoB have significantly advanced our understanding of the regulation of hepatic apoB-containing lipoprotein production. Reconstitution of VLDL assembly and secretion has been successful using cell cultures and is now being attempted using in vitro systems. The use of specific inhibitors of MTP or inhibitors of various proteases will facilitate dissection of the cellular and molecular events involved in apoB secretion and degradation. With efforts to further identify and characterize proteases and other factors involved in ER and post-ER apoB degradation, surprising findings will certainly emerge and eventually reveal whether degradation occurs through a common proteasome-mediated pathway or whether distinct mechanisms are involved in these processes. Understanding how the intracellular degradation of apoB-100 is achieved not only represents an interesting fundamental cell biology question, but also will guide pharmacological strategies to suppress the secretion of apoB-100, a protein that is otherwise constitutively synthesized and potentially atherogenic.

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