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APOLIPOPROTEIN B: mRNA Editing, Lipoprotein Assembly, and Presecretory Degradation

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■ **Abstract** Apolipoprotein (apo)B circulates in two distinct forms, apoB100 and apoB48. Human liver secretes apoB100, the product of a large mRNA encoding 4536 residues. The small intestine of all mammals secretes apoB48, which arises following C-to-U deamination of a single cytidine base in the nuclear apoB transcript, introducing a translational stop codon. This process, referred to as apoB RNA editing, operates through a multicomponent enzyme complex that contains a single catalytic subunit, apobec-1, in addition to other protein factors that have yet to be cloned. ApoB RNA editing also exhibits stringent *cis*-acting requirements that include both structural and sequence-specific elements—specifically efficiency elements that flank the minimal cassette, an AU-rich RNA context, and an 11-nucleotide mooring sequence—located in proximity to a suitably positioned (usually upstream) cytidine. C-to-U RNA editing may become unconstrained under circumstances where apobec-1 is overexpressed, in which case multiple cytidines in apoB RNA, as well as in other transcripts, undergo C-to-U editing. ApoB RNA editing is eliminated following targeting of apobec-1, establishing that there is no genetic redundancy in this function. Under physiological circumstances, apoB RNA editing exhibits developmental, hormonal, and nutritional regulation, in some cases related to transcriptional regulation of apobec-1 mRNA. ApoB and the microsomal triglyceride transfer protein (MTP) are essential for the assembly and secretion of apoB-containing lipoproteins. MTP functions by transferring lipid to apoB during its translation and by transporting triglycerides into the endoplasmic reticulum to form apoB-free lipid droplets. These droplets fuse with nascent apoB-containing particles to form mature, very low-density lipoproteins or chylomicrons. In cultured hepatic cells, lipid availability dictates the rate of apoB production. Unlipidated or underlipidated forms of apoB are subjected to presecretory

degradation, a process mediated by retrograde transport from the lumen of the endoplasmic reticulum to the cytosol, coupled with multiubquitination and proteasomal degradation. Although control of lipid secretion in vivo is primarily achieved at the level of lipoprotein particle size, regulation of apoB production by presecretory degradation may be relevant in some dyslipidemic states.

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INTRODUCTION

Apolipoprotein (apo)B is a large glycoprotein that serves an indispensable role in the assembly and secretion of lipids, including triglyceride and cholesterol of both dietary and endogenous origin, and also in the intravascular transport and receptor-mediated uptake and delivery of distinct classes of lipoproteins. The importance of apoB thus spans a range of functions, from the absorption and processing of dietary lipids to the regulation of circulating lipoprotein levels. This latter property underlies its relevance in terms of atherosclerosis susceptibility, which is highly correlated with the ambient concentration of apoB-containing lipoproteins, principally low-density lipoproteins (LDL).

Mammalian lipoprotein metabolism has evolved a complex partitioning of transport processes for lipid of dietary and endogenous origin, a phenomenon reflected in the existence of two forms of the apoB protein. Referred to as apoB100 and apoB48, each is encoded by a single *APOB* gene. The molecular mechanism responsible for the production of apoB48 in mammals involves a site-specific RNA modification, referred to as C-to-U RNA editing. The first part of this review addresses some of the interesting features of this process and its emerging importance in other areas of RNA biology. Independent of the posttranscriptional modification that yields distinct molecular species of apoB protein, intracellular regulation of apoB assembly and secretion is linked to sequential events in its translation and passage through the endoplasmic reticulum and Golgi apparatus. These events and the associated factors involved in the posttranslational regulation of apoB assembly and secretion form the focus of the second part of this review.

OVERVIEW OF APOB mRNA EDITING

Two forms of apoB exist in mammals. ApoB100 represents the full-length protein containing 4536 residues and is the exclusive form synthesized in human liver (153). The small intestine of all mammals, as well as the liver of certain species, synthesizes apoB48, a protein that is colinear with the amino terminal 2152 residues of the full-length form. ApoB100 is the major protein component of LDL and contains the domain required for interaction of this lipoprotein species with the LDL receptor (153). In addition, apoB100 contains an unpaired cysteine residue, at position 4326, which mediates a covalent interaction with apo(a) and thereby generates another distinct atherogenic lipoprotein, referred to as Lp(a) (18, 82). Because both of these domains are contained within the carboxyl-terminal half of apoB, many of the important functional differences in the metabolism of apoB-containing lipoproteins can be assigned to specific domains that are missing from the smaller protein. In humans, apoB48 circulates in association with chylomicrons and chylomicron remnants, and these particles, by virtue of their content of apoE, are cleared by a distinct receptor referred to as the LDL-receptor related protein (53). There are exceptions to this (oversimplified) paradigm, and functional plasticity exists in lipoprotein recognition and uptake by these receptors, at least in mice (62). Nevertheless, under normal circumstances in human subjects, plasma residence time of chylomicrons is very short (minutes to hours) compared with that of LDL ($\sim$2 days), and thus apoB48 can be viewed as a crucial adaptation by which dietary lipid is delivered from small intestine to liver, whereas apoB100 can be viewed as participating in the transport and delivery of endogenous plasma cholesterol.

In 1987, Powell et al (102) and Chen and colleagues (24) reported the basis by which a common structural gene encoding apoB produces two distinct protein isoforms, a process referred to as RNA editing. A site-specific C-to-U editing reaction produces a UAA stop codon and translational termination of (intestinal) apoB

mRNA at residue 2152, the form referred to as apoB48 (24, 102). This C-to-U conversion, a site-specific hydrolytic deamination, is mediated by a multicomponent enzyme complex containing a single catalytic subunit apobec-1 as well as other, yet unidentified, auxiliary components (48, 125). Considerable insight has been gained over the past decade concerning the molecular genetics and biochemical mechanisms regulating this process, and each has revealed new questions concerning the biology of RNA editing.

MOLECULAR MECHANISMS REGULATING APOB mRNA EDITING

Cis-Acting Elements in ApoB RNA that Constrain C-to-U RNA Editing

C-to-U editing of apoB mRNA is an exquisitely precise reaction that normally targets a single cytidine from the 14,000 bases comprising the spliced nuclear apoB transcript. The requisite *cis*-acting elements for site-specific deamination of apoB RNA are contained within approximately 25 nucleotides flanking the edited cytidine at position 6666 of human apoB cDNA (Figure 1; see color insert) and include a region of 11 nucleotides, located five bases downstream from the edited base, in which virtually all mutations (with the exception of a G^{6677} transversion) reduce or abolish in vitro RNA editing (7, 114). This region, spanning nucleotides 6671–6681, has been referred to as a "mooring sequence" and is indispensable for C-to-U editing of the cytidine at C^{6666} (7, 114). Synthetic RNA templates containing this 25-nucleotide motif will support (at least low levels of) C-to-U editing in vitro (6, 30). Operationally, the minimal requirements for C-to-U editing of an RNA template include the mooring sequence, an upstream C positioned 5 ± 1 nucleotides 5′ to this cassette, and an AU-rich context. Restoration of the levels of C-to-U editing encountered with the natural substrate (i.e. the ∼90% editing of intestinal apoB mRNA) requires additional flanking sequences, whose properties include enrichment with AU residues and the presence of several distinct "efficiency elements," which are contained in the region between nucleotides 6609 and 6747, as illustrated in Figure 1; see color insert (7, 51). The 11-nucleotide mooring sequence cassette also facilitates C-to-U editing of heterologous (e.g. albumin) chimeric RNA substrates that contain an optimally positioned C located upstream (5, 6, 8). It is interesting to note that this mooring sequence, inserted into a heterologous chimeric RNA, was not capable of directing C-to-U conversion of a cytidine placed downstream (i.e. 3′) of this region (97). Accordingly, it was proposed that the orientation of the mooring sequence plays a crucial role in the optimal positioning of the editing machinery with respect to an appropriately positioned, i.e. upstream, target cytidine. Recent findings, however, challenge the exclusivity of this suggestion. Specifically, observations from Yamanaka et al indicate that C-to-U editing of the apoB mRNA in the livers of transgenic mice overexpressing apobec-1 is no longer confined to the canonical C^{6666}, but that

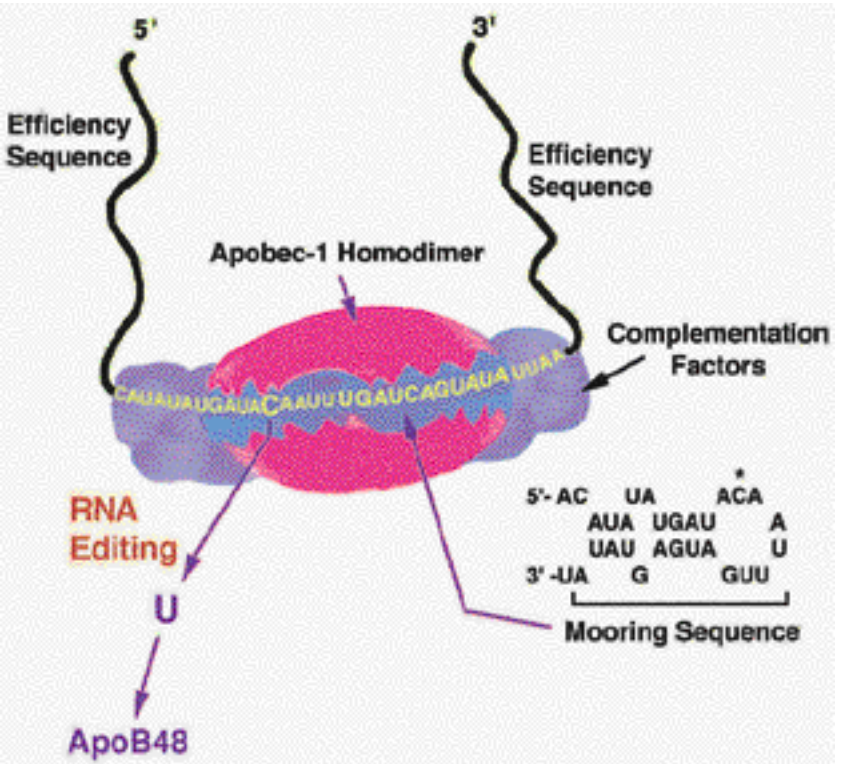

Figure 1 Apolipoprotein B (apoB) mRNA editing; minimal sequence elements and enzymatic machinery. ApoB48 is produced as a result of C-to-U RNA editing of the nuclear apoB transcript. The minimal sequence requirements for this reaction include the specified nucleotides that flank the edited C at position 6666 of apoB RNA. The requisite functional elements include an 11-nucleotide cassette (UGAUCAGUAUA), which is located optimally downstream of the target C. Other requirements include an AU-rich context and the presence of distal efficiency elements both 5' and 3' of the edited C. The region immediately flanking the edited C (*asterisked*) is proposed to form a stem loop structure. The components of the apoB editing enzyme so far identified include apobec-1, which exists as a homodimer, as well as other protein components, referred to as complementation factors. The number and identity of these factors remain unknown.

multiple C residues downstream of the mooring sequence are also edited (148). The most reasonable interpretation of these findings is that physiological editing of apoB mRNA is normally restricted to a single target through mechanisms that include structural constraints, specific sequence requirements, and a fine balance of *trans*-acting factors. There exists a second, minor site in human apoB RNA at position C^{6802}, downstream of the canonical site C^{6666}, which is also a target of physiological C-to-U editing. In this instance, an A<u>C</u>A codon is deaminated to A<u>U</u>A (91). There is no anticipated change, however, in apoB protein sequence with this editing reaction, because editing at the 6802 site is downstream of the anticipated translational stop at U^{6666}. Editing at the C^{6802} site occurs at a lower frequency than that at the canonical site, with ∼20% of cDNAs that contained an edited C^{6666} demonstrating editing at the downstream site (91). Flanking sequences in the vicinity of this second targeted cytidine also play a role in regulating C-to-U editing at this site (51). Physiological editing thus operates within constraints of the target RNA template, including those imposed by its overall composition (AU content), by its secondary structure, and by the directional orientation of a requisite mooring sequence (downstream) of the canonical cytidine nucleotide, as well as other structural elements. Physiological C-to-U editing of apoB also requires a critical stoichiometry of apobec-1 plus other *trans*-acting proteins (discussed below), which are organized in a configuration that permits selection of a single site in the nuclear apoB mRNA. Both sets of constraints are apparently overcome in situations where apobec-1 is overexpressed.

Consequences of, and Lessons from, Hyperediting

C-to-U editing of apoB mRNA is less constrained when apobec-1 is overexpressed. Multiple cytidine nucleotides are targeted for deamination, a condition referred to as hyperediting or promiscuous editing (121, 148). The selection of these additional sites is not random, nor is it processive in nature. These features suggest that hyperediting may reveal important clues to the operational constraints for physiological C-to-U editing and the selection of targets. Distinct clusters of additional Cs located in proximity to mooring or mooring-like sequence elements are preferred targets, but there appears to be no stringent requirement for an exact mooring sequence, unlike editing at the canonical C^{6666} site (148). In addition to the downstream C residues noted to be edited in transgenic mice and rabbits overexpressing apobec-1, upstream C residues are also promiscuously edited in rat hepatoma cells expressing high levels of apobec-1 (121). As alluded to above, there is an alternative site of physiological editing of the human apoB mRNA. The frequency of this editing event increased in the livers of transgenic apobec-1 mice, where up to 75% of the C^{6802} was edited (148). The region flanking the C^{6802} site is AU rich and contains a mooring-like sequence with 6 of 11 matches and a spacer region of 6 rather than 4 nucleotides, which suggests that, like editing at the canonical C^{6666} site, editing at the alternative site also manifests operational requirements (148). Several important principles emerge from studies of hyperediting of apoB mRNA. First, editing efficiency at the additional sites occurs at a

lower efficiency than at the canonical C^{6666} site. Second, although multiple sites are edited, the selection of target sites is not indiscriminate and occurs in proximity to AU-rich and mooring-like sequence elements. Third, although the editing of noncanonical sites can be reproduced in vitro, the enzymatic kinetics are distinct from those of the C^{6666} site. Taken together, analysis of hyperediting reinforces the concept that distinct structural requirements must be present for C-to-U editing to take place. A recent model based on RNase mapping of a 55-mer apoB RNA (Figure 1; see color insert) suggests that the canonical C^{6666} site is positioned within the loop of a stem-loop structure with potential binding sites both 5′ and 3′ (105). An additional concept to emerge from studies of hyperediting and from the modeling alluded to above is that alternative targets of C-to-U editing might exist beyond apoB mRNA. Mooring and mooring-like sequences have been found extensively in the databases and raised the formal possibility that other RNAs may be edited under conditions where apobec-1 expression is favorable. This possibility was directly demonstrated in transgenic mice overexpressing apobec-1 in association with editing of a novel translational repressor mRNA, NAT-1 (146). It is of interest also that these transgenic mice overexpressing apobec-1 at high levels also developed hepatic dysplasia and carcinomas, which suggests a sinister gain of function in association with hyperediting (145). In addition, one group has reported that NF-1 mRNA undergoes C-to-U editing in human tissues, with higher levels of editing noted in tumor versus control tissue (19, 118). Whether this process is related to apobec-1 expression remains unproven.

COMPONENTS OF THE APOB RNA EDITING MACHINERY

As alluded to above, C-to-U editing of apoB mRNA requires protein factors that include apobec-1, in addition to other components (48). These components assemble into a holo-enzyme that has been referred to as an editosome, a term that represents the functional enzyme complex (119).

Characterization of Apobec-1

Apobec-1 is a 27-kDa protein cloned first from rat enterocytes and later from human, mouse, and rabbit small intestine (46, 70, 125). Apobec-1 is a cytidine deaminase and exhibits important structural and biochemical characteristics of this multigene family (90). The primary sequence contains a signature motif of zinc-coordinating residues, specifically His-Val-Glu-$X_{(24–30)}$-Pro-Cys-X-X-Cys, that encompass the active site. As do other cytidine deaminases, apobec-1 functions as a homodimer, although its multimeric form within the context of an intact editing complex remains unknown. Apobec-1 will deaminate a monomeric nucleoside substrate in vitro, and its activity is inhibited by chemical agents known to interfere with other cytidine deaminases, such as tetrahydrouridine (80). In addition to its enzymatic activity, apobec-1 is an RNA binding protein (3, 88).

Moreover, its RNA binding activity is indispensable to RNA editing, a function independent of its actions as a cytidine deaminase. Specifically, all mutations that abolish RNA binding will eliminate RNA editing activity, but some of these mutations leave cytidine deaminase activity relatively well preserved (3, 88, 89). Thus, apobec-1 evolved distinct functions in regard to RNA binding and cytidine deaminase activity, and an understanding of these evolutionary pressures may illuminate the adaptation to deamination of a specific cytidine within apoB mRNA.

Other *Trans*-Acting Factors Involved in Enzymatic Deamination of ApoB mRNA

As alluded to above, apobec-1 exhibits an absolute requirement for additional protein components in order to perform site-specific C-to-U deamination of apoB RNA. Over the past several years, many candidate proteins have been proposed to function in this capacity. In contrast to apobec-1, which in humans is virtually confined to the small intestine (46, 70), the distribution of the remaining protein factors appears widespread, including tissues that express neither apobec-1 nor ApoB mRNA (126, 147). Evidence for a multicomponent enzyme complex emerged from studies of the behavior of rat liver extracts prepared on glycerol gradients, where editing activity was associated with a complex of 11S mobility that increased to 27S (48, 119). These findings are at odds with other reports that the editing enzyme activity from intestinal extracts exhibits a minimal molecular mass of ∼125,000 (29). Several reports have demonstrated the presence of proteins that bind to apoB RNA from preparations of editing extracts, including those fractionated through glycerol gradients, those isolated from cytosolic S100 extracts, and those prepared from isolated nuclear extracts (69, 83, 92, 126, 150). Proteins predominantly in the 40- to 66-kDa range have been identified in this manner from a number of tissue sources, as have larger proteins, in the size range of 100 and 240 kDa, which have been identified from editosomal complexes (108, 150). Other proteins, including ABBP-1 and hnRNP-C, have been identified through a two-hybrid screen, although their role in apoB mRNA editing remains to be fully revealed (44, 71). Thus, although a number of candidates have been identified through potential interactions with apobec-1 and also with apoB RNA itself, only one, discussed below, has been demonstrated to complement apobec-1 in a reconstituted apoB RNA editing assay (84). Mehta & Driscoll (84) have identified a 65-kDa protein, isolated through RNA affinity chromatography as an apoB RNA-specific binding activity, that appears on silver-stained gels to be an almost homogeneous protein species. Preparations of this material effectively complement recombinant apobec-1 in restoring editing activity, and it represents a plausible candidate complementation factor. In addition, the apparent RNA-binding specificity of this preparation suggests that this component of the editing machinery may play a role in constraining the site selection of apobec-1 (84). The cloning and formal identification of this 65-kDa factor is eagerly awaited.

NUTRITIONAL REGULATION OF APOB RNA EDITING

ApoB mRNA editing is subject to regulation through a variety of stimuli, including developmental cues and hormonal manipulation as well as nutritional and environmental influences (10, 49, 68, 79, 101, 123, 128, 129, 133). In such considerations, it bears emphasizing that much of the data concerning regulation of apoB mRNA editing has come from studies of rat and mouse liver. As alluded to above, the liver of rats and mice secretes both apoB100 and apoB48 in proportions that are subject to modification through apoB RNA editing. Intestinal apoB RNA editing is developmentally regulated in humans, with early fetal small intestine expressing predominantly unedited apoB mRNA and secreting apoB100 (99, 128). An adult pattern of apoB RNA editing (i.e. >90% apoB48) is established by the third trimester of pregnancy, and there appears to be little regulation of this process after birth (128). Specifically, human intestinal apoB mRNA editing is not responsive to alterations in lipid flux or triglyceride (TG) feeding (78). Similar findings were demonstrated in rat and mouse intestine (54, 65). Although a definitive resolution concerning the composition of the holo editing enzyme will clarify the molecular mechanisms regulating apoB RNA editing, findings from studies in rat and mouse liver illustrate certain important principles.

Genetic and Functional Characterization of Apobec-1

Although it was clear that apobec-1 plays a central role in apoB mRNA editing, formal proof that apobec-1 is the sole catalytic component of the apoB mRNA editing enzyme emerged from studies of *apobec-1*$^{-/-}$ mice. Such mice have been generated by several groups and have been shown to express exclusively apoB100 in the intestine and liver, the result of a complete absence of apoB mRNA editing (57, 85, 86, 95). Despite the presence of LDL containing only apoB100, total plasma cholesterol levels in these mice are only barely elevated (57, 85, 86, 95). Further breeding into *ldlr*$^{-/-}$ mice resulted in a line of double-knockout (*ldlr*$^{-/-}$, *apobec-1*$^{-/-}$) animals expressing no LDL receptors and whose liver and intestine secrete apoB100 exclusively (103). These animals manifest spontaneous hypercholesterolemia on a low-fat diet and develop severe atherosclerosis without dietary supplementation with cholesterol or cholate (103). In this regard, the *apobec-1*$^{-/-}$, *ldlr*$^{-/-}$ mice represent an authentic animal model of human familial hypercholesterolemia. Accordingly, apoB mRNA editing has an enormous impact on the nutritional modulation of plasma apoB levels, because the natural ability of mouse liver to secrete apoB48 in addition to apoB100 permits lipoprotein particle uptake by both LDL receptors and other (i.e. LDL-receptor related protein) dependent pathways that depend on the particle recognition through binding of apoE (62). The absence of apoB mRNA editing from the liver of humans and other mammals whose lipoprotein profile is predominantly LDL based, including rabbits, manifest predictable hypercholesterolemia when there is haploinsufficiency of LDL

receptor expression. By way of example, the ability of mouse liver to edit apoB mRNA presumably accounts for the rather mild hypercholesterolemia found in *ldlr*$^{-/-}$ mice, because the presence of apoB48 in these animals permits lipoprotein uptake through apoE-dependent mechanisms that involve LDL-receptor related protein (53, 62).

Transcriptional and Translational Regulation of Apobec-1 Gene Expression

ApoB mRNA editing is developmentally regulated in association with a transcriptional increase in steady state levels of apobec-1 mRNA abundance (38). Nutritional modulation of apoB mRNA editing, in association with starvation and subsequent carbohydrate feeding in rats, is also associated with an increase in apobec-1 mRNA abundance (38). In this situation, the combination of fasting and refeeding with a high-carbohydrate diet induces hepatic TG accumulation in association with an increase in hepatic apoB mRNA editing (10, 49). Accordingly, animals subjected to this nutritional stress develop fatty liver in association with an increase in the proportion of apoB48 synthesized, relative to apoB100 (10). The survival advantage of this adaptation is unclear, however, because apoB100 and apoB48 appear to function interchangeably with respect to TG secretion, as evidenced by the lack of an apparent phenotype in intestinal lipid absorption and secretion in *apobec-1*$^{-/-}$ mice, where the intestine secretes exclusively apoB100 (57, 85, 86, 95). Recent studies have demonstrated that insulin increases steady state levels of apobec-1 mRNA in isolated rat hepatocytes in vitro, which suggests that there may be additional mechanisms involved in the coordinate regulation of hepatic lipid metabolism and apoB mRNA editing (129, 133). Other situations in which apoB mRNA editing appears to be regulated, for instance following ethanol ingestion, are not associated with changes in the abundance of apobec-1 mRNA (68, 132). In these situations, it is presumed that changes in apoB mRNA editing reflect an alteration in complementation activity. Apobec-1 gene expression is subject to a complex pattern of regulation, which includes the use of alternative promoter sites in mice and rats, coupled with differential alternative splicing (43, 56, 87). In humans, *APOBEC-1* transcription proceeds from a single cluster of start sites, although no functional promoter has yet been identified (37, 55). In addition, human *APOBEC-1* gene expression is regulated in the small intestine through alternative splicing, a process that results in exclusion of exon 2 and introduces a frame shift in the coding region that results in the production of a novel 36–amino acid peptide, referred to as apobec-T (37, 55). This latter peptide lacks the functional domains in apobec-1 that mediate enzyme activity and RNA binding, and its potential function remains unknown. Increased levels of this alternative splice variant were found in human colon cancer samples, and conditional expression of the peptide in cell culture was associated with an alteration in growth, which suggests a potential role unrelated to RNA editing (72).

OVERVIEW OF APOB ASSEMBLY

Kinetics of Lipoprotein Assembly

The initial association of apoB with lipid occurs during its translation by membrane-bound ribosomes (13). The kinetic coupling of translation and lipidation may be due to the tendency of apoB to aggregate in unlipidated form. By adding lipid to apoB cotranslationally, its lipophilic domains avoid nonspecific interactions with themselves, other proteins, and intracellular membranes, thereby ensuring efficient assembly. The product of the cotranslational phase of apoB assembly is a small, dense emulsion particle with a maximum diameter of ~25 nm (122). The formation of large heterogeneous very low-density lipoproteins (VLDL) in liver (30–80 nm diameter) or chylomicrons in intestine (75–1200 nm diameter) is thought to occur via a second, posttranslational step in which preformed, apparently protein-free TG droplets fuse with the nascent apoB-containing particles formed during translation. The co- and posttranslational association of apoB with lipid is commonly referred to as the two-step model of lipoprotein assembly (2, 14, 122, 124).

Role of MTP and Other Protein Cofactors

In addition to the ubiquitous protein and lipid biosynthetic and trafficking proteins present in most cells, the assembly of apoB with lipid requires a dedicated endoplasmic reticulum (ER)-localized cofactor termed the microsomal triglyceride transfer protein (MTP). MTP is a heterodimer consisting of a 97-kDa unique subunit complexed with the ubiquitous ER-localized chaperone, protein disulfide isomerase. This factor may function in a variety of ways; however, based on its ability to transfer lipid from donor to acceptor vesicles in vitro, it is proposed (41) that MTP transfers lipid from the ER membrane or some other donor site to apoB during the cotranslational phase of lipoprotein assembly. Several studies have demonstrated a physical interaction between apoB and MTP (60, 61, 98, 144), and at least one inhibitor of apoB secretion has been identified that disrupts MTP binding to apoB without affecting lipid transfer activity in vitro (61). In addition, mutational disruption of an MTP binding site in apoB inhibits lipoprotein secretion (16). Hence, MTP-apoB interactions may be critical to achieve net transfer of lipid to apoB during its translation.

In some but not all studies, MTP has been shown to play a role in the second step fusion of nascent apoB-containing particles with TG droplets (40, 136). This point has been clarified by two studies demonstrating that MTP may be necessary for the trafficking of TG from the cytosol to the ER lumen, a process that occurs independently of apoB (47, 104, 137). Therefore, although MTP may not play a direct role in the fusion of lumenal TG droplets with apoB during the second step of assembly, it is necessary for the formation of both precursor particles.

In addition to MTP, other cofactors may be required for apoB assembly. The autosomal recessive disorder, chylomicron retention disease, is characterized by a selective defect in chylomicron production with little impact on hepatic VLDL (67).

The underlying defect in this disease is unknown; however, its inheritance is unlinked to all candidate genes studied to date, including the intestinal apolipoproteins, MTP, and fatty acid binding proteins 1 and 2 (24a, 100). In addition, normal apoB mRNA editing (97) and MTP expression (24a, 138) were detected in intestinal biopsies. Hence, an additional factor may exist that is specifically required for the intracellular formation, maturation, or trafficking of apoB48-containing lipoproteins in the intestine. Finally, it should be noted that the mouse mammary tumor cell line, C127, assembles and secretes transfected apoB41 in the apparent absence of MTP expression (52). Characterization of this MTP-independent assembly pathway may reveal the existence of other factors that play a role in the intracellular processing of apoB.

Lipid Biosynthesis and Mobilization

Most enzyme systems involved in the biogenesis of the lipid components of apoB-containing lipoproteins have been implicated in modulating apoB assembly and secretion (28). The coupling of cytosolic TG to apoB assembly may require hydrolysis followed by reesterification by acyl-coenzyme A:diacylglycerol acyltransferase (DGAT) (36, 39, 149). A candidate TG hydrolase from pig liver has been identified and characterized (73) and a DGAT enzyme was recently cloned based on sequence similarity with the cholesterol esterifying enzyme acyl-coenzyme A:cholesterol acyltransferase (ACAT) (21). Although only one DGAT gene has thus far been identified, biochemical evidence suggests the existence of two forms of this ER-localized membrane protein, one whose active site faces the cytosol and one that faces the ER lumen (96). Likewise, two forms of ACAT have been identified that display different tissue distributions and possibly inverted topologies at the level of the ER membrane (4). ACAT-1 (23) is ubiquitously distributed in tissues and may esterify cholesterol on the cytoplasmic side of the ER membrane, consistent with a role in intracellular cholesterol homeostasis. ACAT-2 is most abundant in liver and intestine and may be involved in the deposition of cholesteryl ester in the ER lumen (4, 20, 94). It is an intriguing possibility that the lumenal forms of DGAT and ACAT are specifically involved in the mobilization of lipids for apoB-containing lipoprotein assembly. Finally, Du et al (31, 135) have suggested a regulatory role for the enzyme 7α-hydroxylase and oxysterols in the assembly and secretion of apoB-containing lipoproteins, a phenomenon that may be mediated through sterol response element binding protein 1. In CHO cells, the proper processing of apoB into a secretable lipoprotein appears to require the activities of both MTP and 7α-hydroxylase (35).

Lipid Binding Properties of ApoB

The basic lipid binding structures in apoB may consist of a combination of amphipathic α-helical and β-strand domains (112) (Figure 2; see color insert). The β domains in apoB may exist in the form of amphipathic belt-like structures, the

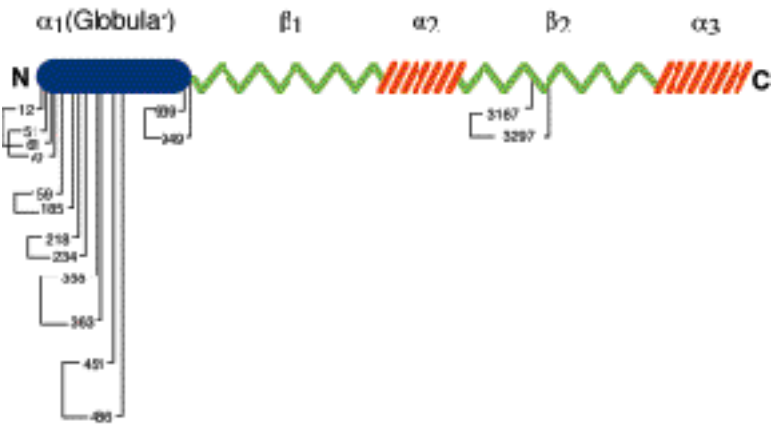

Figure 2 Domain structure of apolipoprotein (apo) B100. The strong lipid binding properties of apoB reside within two domains predicted to form amphipathic β-strands (β1 and β2) and two domains rich in amphipathic α-helical structures (α2 and α3) (112). The α1 globular domain, which is heavily disulfide bonded (*number* and *brackets*, location of Cys residues involved in disulfide bonding), displays amino acid sequence similarity with lipovitellin and the amino-terminal domain of MTP. Although this domain does not support lipoprotein assembly when expressed on its own, the α1 domain is required for the initiation of lipoprotein assembly.

hydrophobic face of which could interact irreversibly with the neutral lipid core (110, 112, 113). The α-helical domains are similar to those found in the soluble class of apolipoproteins, such as apoA-I and apoE, and may confer avid but reversible binding to the lipoprotein surface. The predicted lipid binding domains of apoB are localized primarily within the C-terminal 80% of the polypeptide chain. In contrast, the amino-terminal ~20% of apoB (α_1 domain) is globular, highly disulfide bonded, and is secreted, from both hepatic and nonhepatic (MTP negative) cells, in a lipid free form (42, 151). Although the α_1 domain is incapable of forming a lipoprotein on its own, proper folding of this domain in transfected forms of apoB28 and apo50 as well as endogenous apoB100 in HepG2 cells is essential for the initiation of MTP-dependent lipoprotein assembly (45, 58, 64, 116, 131).

Clues as to the function of the α_1 domain have emerged from structural studies indicating that it, as well as the amino-terminal domain of the 97-kDa subunit of MTP, displays amino acid sequence similarity with members of the vitellogenin gene family (9, 117). Based on the known crystal structure of lamprey lipovitellin (the processed form of vitellogenin), the α_1 domain of apoB and the amino-terminal domain of MTP comprise an N-terminal globular β-barrel and an extended C-terminal α-helical domain. These domains, which serve as homodimerization surfaces in lipovitellin, may underlie the capacity of MTP to heterodimerize with protein disulfide isomerase (PDI) and to interact transiently with apoB (81). It is interesting to note that the C-terminal lipid binding structures present in lipovitellin are truncated in both apoB and MTP. Segrest et al (111) have proposed that apoB-containing lipoprotein assembly is initiated by the association of MTP with the α_1 domain of apoB, a process that could reconstitute a triangular lipid-binding pocket similar to that found in lipovitellin. Although the exact mechanism responsible for apoB's initial lipid acquisition is not known, it is clear that translation of the amino terminal ~25% of apoB in combination with MTP is sufficient to form a small neutral lipid core–containing lipoprotein. This nascent particle is then expanded by both co- and posttranslational mechanisms to form a mature VLDL or chylomicron.

POSTTRANSLATIONAL REGULATION OF APOB SECRETION

Presecretory Degradation of ApoB in Cultured Hepatic Cells

The formation of apoB-containing lipoproteins is a particularly complex example of secretory protein biogenesis and, therefore, carries the potential for the production of misfolded and otherwise aberrant lipoprotein products. Perturbed folding and assembly of secretory precursor proteins within the ER usually results in retarded or blocked anterograde transport. Misfolded proteins are ultimately disposed of by a process involving retrograde transport via the Sec61p translocation channel from the ER lumen to the cytosol, followed by multiubiquitination and proteasomal degradation (17).

The first observation of presecretory apoB degradation was made by Borchardt & Davis (12), who demonstrated by metabolic pulse-chase analysis that a large proportion of newly synthesized apoB in cultured rat hepatocytes was not recovered from cell pellets or media. Similar observations have been made in other cell systems, including the human hepatoma cell line HepG2 (27) and McA-RH7777 cells, a rat hepatoma line (140). In HepG2 cells, as much as 85% of newly synthesized apoB is degraded prior to secretion (27). In hepatoma cells, stimulation of TG synthesis by addition of sodium oleate dramatically increases apoB secretion without affecting its rate of synthesis (27, 140). These and other studies gave rise to a paradigm in which apoB is synthesized in excess and shunted to a pathway involved in particle assembly and secretion or presecretory degradation. The balance between these two pathways, which ultimately determines apoB production rates, is dictated largely by lipid availability (28).

Presecretory Degradation of ApoB In Vivo

Although substantial evidence suggests that apoB production can be dramatically modulated by lipid availability in hepatic cells in culture, considerably less evidence points to this as a common mode of regulation in normal human or animal physiology. Both short- and long-term feeding of diets containing as much as 30% TG by weight had no impact on apoB48 production by jejunal and ileal enterocytes, relative to diets containing no fat (25). Similar alterations in hepatic VLDL TG and cholesterol ester production have been observed without corresponding changes in hepatic apoB production (67, 109, 120). These results strongly suggest that lipid secretion by the enterocyte and hepatocyte is controlled primarily at the level of particle size and that sufficient TG synthesis occurs, even in the absence of dietary fat absorption, to maintain a constitutive level of apoB secretion.

Although the hepatic and intestinal secretion of apoB may be constitutive under most circumstances, it is possible that control of apoB production at the level of presecretory degradation is important in some dyslipidemic states, particularly those characterized by hepatic overproduction of apoB, such as occurs in familial combined hyperlipidemia (28, 66, 127). Another condition under which apoB production may be affected by presecretory degradation is abetalipoproteinemia, a disease caused by genetic lesions in the gene for the 97-kDa subunit of MTP (115). Clearly, without the ability to transfer lipid to apoB either during or after its initial synthesis, apoB becomes extremely labile and is subjected to intracellular proteolysis. Presumably, a similar outcome occurs in animals treated with inhibitors of MTP. For example, treatment of Watanabe (LDL receptor deficient) rabbits with an MTP inhibitor normalized plasma cholesterol and TGs (139), a phenomenon that is likely accompanied by extensive presecretory degradation of apoB. Hence, rates of apoB-dependent lipid secretion may be determined both by particle size and, under some circumstances, presecretory degradation. As such, the mechanisms underlying both the assembly and degradation of apoB represent potential control points for impacting apoB production rates.

MECHANSIMS OF APOB DEGRADATION

Translocation of ApoB Across the Endoplasmic Reticulum Membrane

The most highly cited mechanism proposed to account for the hepatic regulation of presecretory apoB degradation centers around the translocation of apoB across the ER membrane. The translocation of most secretory precursor proteins across the ER membrane in higher eukaryotes is believed to occur cotranslationally via a tight junction between the ribosome and the ER membrane. Although the ribosome-membrane junction may be dynamic (50, 93), it appears by most criteria that once initiated, polypeptides chain translocation into the ER progresses to completion (76). Early studies on apoB, however, indicated that its complete translocation may be dependent on ongoing assembly with lipids. This hypothesis arose from studies in a variety of hepatic cell systems in which apoB was judged to be accessible to proteases and antibodies added to microsomes or permeabilized cells (26, 32, 33, 141). In addition, a large population of apoB resisted extraction from microsomes with alkaline pH buffers, a characteristic of integral membrane proteins (26, 106). Subsequent studies suggested that MTP may be required for apoB translocation, as virtually no transfected apoB53 appeared to undergo complete translocation in CHO cells, an MTP-negative cell line (130).

Delivery of ApoB to the Proteasome by Translocation Arrest

A model based on the apparently inefficient translocation of apoB has emerged. Translocation of the first ~85 kDa of apoB is believed to occur efficiently and independently of MTP. However, in the absence of MTP or sufficient secretion-coupled lipid, the translocation of apoB downstream of the 85-kDa domain is disrupted (32). No consensus exists as to the exact sites within apoB responsible for translocation arrest. Translocation arrest sites have been proposed to reside within the α_1 (15, 81), β_1 (75), and β_2 (22) domains of apoB (Figure 2). If the translocation-arrested apoB persists, it is subjected to multiubiquitination and degradation by the proteasome, a process that is facilitated by cytoplasmic Hsp70 (34, 152, 155). Although translocation-arrested apoB was initially thought to be a terminal intermediate in apoB degradation (26), other studies suggested that transmembrane apoB can be posttranslationally transported into the ER by a process of "lipid facilitated translocation" (107).

Delivery of ApoB to the Proteasome by Retrograde Transport from ER Lumen to Cytosol

In contrast to the model in which lipid availability and MTP control translocation of apoB, others have found that apoB translocation requires neither MTP nor ongoing lipoprotein assembly. Evidence for complete unregulated translocation of apoB is based on a considerable body of literature, some of which is outlined below. First, protease protection experiments demonstrating large amounts of cytosolic

apoB have not been reproduced in all cases. In one study, the relative amount of trypsin-accessible apoB100 in HepG2 cell microsomes was less than 20%, a value similar to that observed for two secretory control proteins (63, 74). Second, the concept that lipid facilitates translocation of apoB across the ER membrane has never been demonstrated directly. On the contrary, the same extent of protease accessibility of apoB was observed in control and oleate-stimulated HepG2 cells, despite the fact that oleate causes a several-fold increase in apoB assembly and secretion (putatively by enhancing movement of apoB into the ER lumen) (33). Third, carbonate-resistant apoB, which was previously thought to represent transmembrane protein, was found to be fully extractable by including low concentrations of deoxycholate, a condition that fails to extract bona fide integral membrane proteins (106). This population of apoB was found to be composed primarily of small, underlipidated particles with densities in the high-density lipoprotein range. Finally, studies in rat hepatocytes showed that carbonate-resistant (membrane associated) apoB was more highly mannosylated than secreted apoB was (142). As the mannose content of secretory proteins is generally reduced by mannosidases during transport through the secretory pathway, this is the expected result for most generic secretory proteins. However, if the membrane-associated apoB failed to translocate fully, most of the potential sites for N-linked glycosylation would be underutilized, leading to a lower mannose content. This finding was extended recently by the demonstration that all apoB, whether destined for assembly and secretion or intracellular degradation, is fully N-glycosylated at sites positioned well downstream of all domains that have been reported to promote translocation arrest (59). As the oligosaccharyltransferase active site resides in the ER lumen, these data are difficult to reconcile with models proposing that the forward translocation of apoB is disrupted to any appreciable extent.

Complete translocation of apoB into the ER suggests that its multiubiquitination and proteasomal degradation must require retrograde translocation. It is proposed that in hepatoma cells, the predominant substrate for intracellular degradation is an aberrant underlipidated particle whose surface is too small to accommodate the folding of the entire apoB protein. The misfolded apoB on the surface of these particles may account for their tendency to associate with the inner leaflet of the ER membrane in a manner that resists carbonate extraction. These particles may undergo one of two fates: (*a*) posttranslational expansion of the lipid core and surface by MTP, followed by release from the inner leaflet of the ER membrane and secretion, or (*b*) retrograde transport from the ER lumen to the cytosol, a process that is mechanistically coupled to multiubiquitination and proteasomal degradation (Figure 3; see color insert).

Cotranslational Degradation of ApoB and Other Proteolytic Systems

In addition to full-length apoB, nascent chains of apoB are targeted for degradation by the ubiquitin-proteasome pathway (11, 77, 154). However, proteasomal degradation is observed only for those forms of apoB that are ≥65% complete (11). As

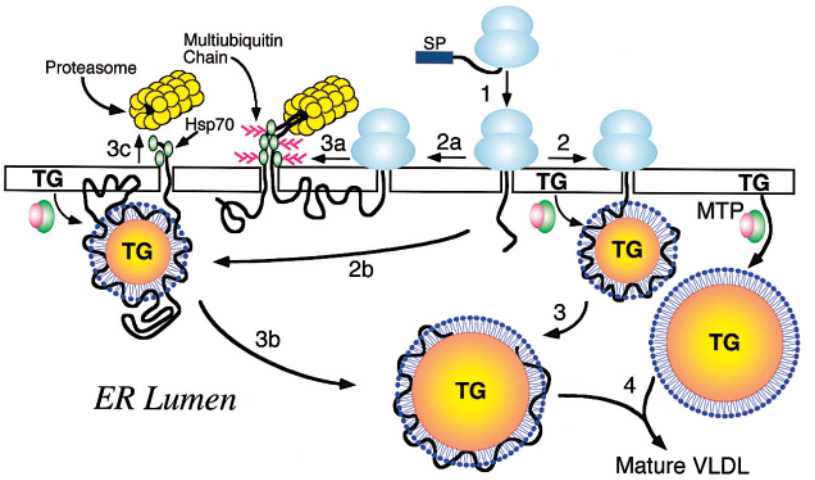

Figure 3 Assembly and degradation of apolipoprotein (apo) B-containing lipoproteins. The apoB signal peptide (SP) targets apoB-synthesizing ribosomes to the endoplasmic reticulum (ER) membrane (*step 1*). Translation of the amino-terminal ~25% of apoB in combination with microsomal triglyceride transfer protein (MTP) created a small, dense emulsion particle (*step 2*) that continues to be enlarged during translation. Upon release from the ribosome (*step 3*), the lipoprotein precursor fuses with triglyceride (TG) droplets originating in the smooth ER (step 4). This fusion reaction gives rise to mature very low-density lipoproteins (VLDL) in the liver and chylomicrons in the intestine. In the absence of MTP, apoB is translated without undergoing lipidation (*step 2a*). This population is rapidly degraded, sometimes prior to the completion of translation, by a process of retrograde translocation from the ER lumen to the cytosol coupled to addition of multiubiquitin chains and proteasomal degradation (*step 3a*). Under conditions of limiting MTP or lipid, aberrant, underlipidated particles are generated that are too small to accommodate the proper folding of apoB (step 2b). The unfolded domains of apoB may interact with the inner leaflet of the ER membrane. These particles can be lipidated posttranslationally and released from the ER membrane (step 3b) or targeted for proteasomal degradation (step 3c). Both co- and posttranslational proteasomal degradation of apoB are dependent on Hsp70.

it takes ~9 min to translate 65% of apoB100 in HepG2 cells (15), it is possible that some misfolded apoB is recruited for retrotranslocation even before translation is complete. This extremely rapid, cotranslational degradation pathway may be used for apoB that is completely devoid of lipid and hence particularly labile (Figure 3). Although the ubiquitin-proteasome pathway appears to be a major route for the disposal of misfolded apoB, it should be noted that other proteolytic systems located in both the ER (1, 143) and post-ER compartments (134) may also control the presecretory degradation of apoB.

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