

Assembly and secretion of lipoproteins

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1. Overview of lipoprotein secretion into the circulation

Lipoproteins are secreted into the circulation from hepatocytes of the liver and enterocytes of the intestine. All plasma lipoproteins share a common structure consisting of a neutral lipid core of triacylglycerols (TGs) and cholesteryl esters surrounded by a surface monolayer of phospholipids, unesterified cholesterol and specific proteins called apolipoproteins (Fig. 1 and Chapter 18). The primary function of plasma lipoproteins is to transport hydrophobic, water-insoluble lipids in the circulation. The TG-rich lipoproteins deliver TGs made in the liver and intestine to other tissues in the body for either storage or utilization as an energy source. High-density lipoproteins are thought to remove cholesterol from peripheral tissues (i.e. tissues other than liver) and deliver it to the liver for excretion in bile in a process referred to as ‘reverse cholesterol transport’ (Chapters 16, 20). A high level of plasma lipids is a strong risk factor for development of cardiovascular disease (Chapter 22). The steady state level of lipoproteins in the cir-

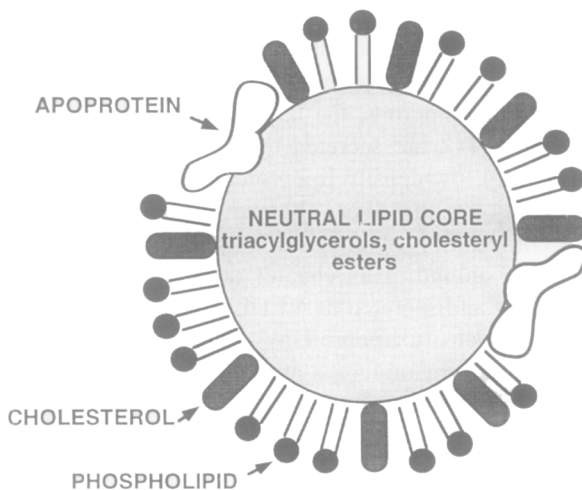


Fig. 1. Generalized structure of a lipoprotein particle. Lipoproteins are approximately spherical particles that consist of a neutral lipid core (triacylglycerols and cholesteryl esters) surrounded by a surface monolayer of amphipathic lipids (unesterified cholesterol and phospholipids) and specific apoproteins. Courtesy of Dr. J. Segrest, University of Alabama, with permission.

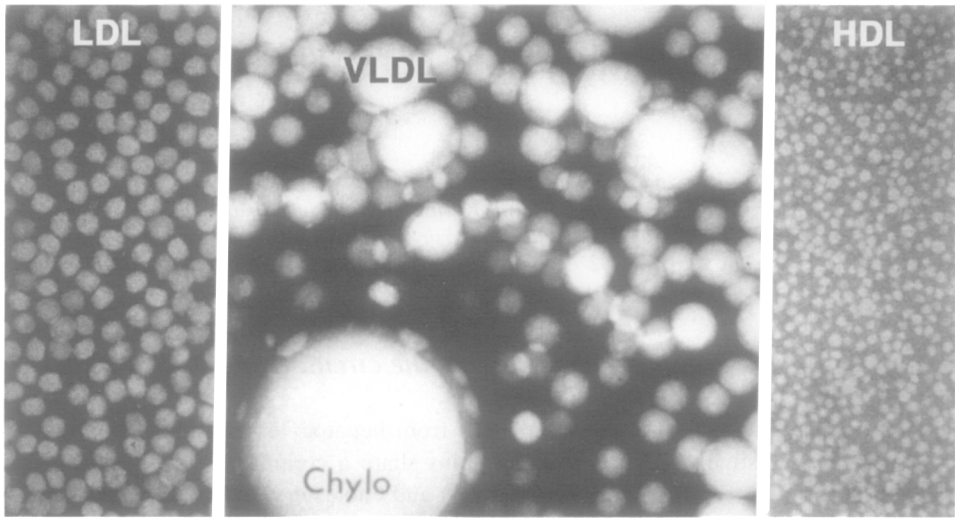


Fig. 2. Negative staining electron micrographs of human plasma lipoproteins (diameters 10–1000 nm). The largest particles [chylomicrons (Chylo) and VLDLs] contain a higher ratio of lipid to protein, and are therefore less dense, than low-density lipoproteins (LDLs) and high-density lipoproteins (HDLs) which contain relatively more protein. Photograph courtesy of Dr. R. Hamilton, University of California at San Francisco, with permission.

culatation results from a balance between their rates of secretion into, and removal from, plasma. This chapter focuses on the mechanisms by which lipoproteins are assembled and secreted from the liver and intestine. The synthesis of the constituent lipids of the lipoproteins is described in Chapters 8 (phospholipids), 15 (cholesterol) and 10 (TGs).

The major plasma lipoproteins (Fig. 2) are usually classified according to density (table 1 in Chapter 18). Since lipids have lower buoyant densities than proteins, lipoproteins that have a high ratio of lipid to protein have lower density than lipoproteins having a lower ratio of lipid to protein. Chylomicrons, the largest and most lipid-rich particles, whose major lipid component is TG, are secreted by the intestine and are abundant in plasma only after a meal. Very low-density lipoproteins (VLDLs), which are also rich in TG, are secreted mainly by the liver, although some are also of intestinal origin. The size and lipid composition of chylomicrons and VLDLs vary according to the nutritional status of the animal. This chapter discusses how lipids are assembled with apolipoprotein (apo) B and secreted as VLDLs and chylomicrons. Intermediate-density lipoproteins and low-density lipoproteins (LDLs) are generated in the circulation by lipolysis of TGs within chylomicrons and VLDLs. High-density lipoproteins are particles of diverse composition that are formed in the circulation (Chapter 20).

Apo B is an essential component of chylomicrons, VLDLs and low-density lipoproteins. Unlike the ‘exchangeable’ plasma apolipoproteins (e.g. apos E, A1, A2, C), apo B does not exchange among lipoproteins and is present in plasma only when associated with lipids. In addition to apo B, VLDLs and chylomicrons contain apo E and apos C, and chylomicrons also contain some apos A1 and A2.

In many respects, the secretion of apo B from cells follows a pathway identical to that of typical secretory proteins. The mRNA encoding apo B is translated on ribosomes bound to the endoplasmic reticulum (ER) and the protein is translocated across the ER membrane into the lumen. A unique difference between apo B and other secretory proteins is that apo B must be non-covalently associated with lipids before secretion. Newly formed apo B-containing lipoproteins undergo vesicular transport from the ER through the Golgi stacks. Secretory vesicles, containing lipoproteins, are formed in the *trans*-Golgi network and subsequently fuse with the plasma membrane releasing apo B-containing lipoproteins from the cell. Evidence that apo B transits the same secretory route as other secretory proteins was provided by electron microscopy studies of rat liver in which lipid droplets the size of VLDL were detected in the lumen of the ER and Golgi [1]. In cultured rat hepatocytes, synthesis of an apo B molecule takes 7 to 15 min and apo B appears in the culture medium 30 to 40 min later. Pulse-labeling experiments indicate that the slow step in passage of apo B through the secretory pathway is movement of the protein out of the ER (R.A. Davis, 1987).

2. Structural features of apolipoprotein B

Full-length human apo B, designated as apo B100, is one of the largest single polypeptide chains known with 4536 amino acids (M_r 513,000) [2]. In humans, the liver secretes exclusively apo B100 whereas the intestine secretes primarily a truncated form of apo B100, called apo B48 (M_r 264,000), along with some apo B100 (J.P. Kane, 1983). Rodent livers, in contrast, secrete both apo B100 and apo B48. A convenient 'centile' nomenclature for apo B variants is commonly used in which apo B species are designated by a number indicating the size of the molecule relative to full-length apo B100. Therefore, in this shorthand scheme, apo B48 refers to the N-terminal 48% of apo B100 and apo B15 refers to the N-terminal 15% of apo B100.

Apo B100 is an amphipathic protein. Some of the complex molecular interactions required for assembly of this protein with lipids can be inferred from its unique domain structure. Indeed, apo B is the only protein known to require association with a lipid microemulsion for secretion. Each VLDL particle contains a single molecule of apo B (J. Elovson, 1988). Computer-based structural analysis of apo B100 predicts a pentapartite structure (Fig. 3) (R. Nolte, 1994) [3] consisting of a globular N-terminal domain followed by alternating amphipathic β -sheets and α -helices. As much as 40–50% of apo B100 is β -sheet structure, which is concentrated in two large regions. At least 25% of the molecule consists of α -helices which are too short to span a membrane. It is likely that the surface of a VLDL particle does not contain sufficient phospholipid molecules to completely cover the surface and, consequently, apo B is probably in direct contact with some TG in the particle core. The β -strands are thought to be the major motif mediating association with lipids. Since the amphipathic β -sheets of apo B have both polar and non-polar faces, the prediction is that the non-polar face interacts with neutral lipids, such as TGs, whereas the polar face is exposed to the aqueous environment.

A general correlation exists between the length of the apo B molecule and its ability

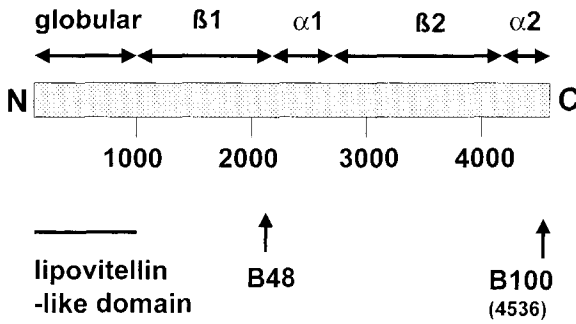


Fig. 3. Schematic representation of the pentapartite structure of apo B100. The N-terminal globular domain and the alternating α and β domains are indicated above the representation of the polypeptide. Numerical values below the polypeptide indicate an amino acid residue scale. Human apo B100 contains 4536 amino acids; the size of apo B48 is indicated. The N-terminal 1000 amino acid residues of apo B100 are homologous with the lipid-binding pocket of lamprey lipovitellin.

to associate with lipids. Naturally occurring mutations in the human apo B gene, in the group of diseases known as hypobetalipoproteinemia, show that secretion of some truncated forms of apo B as lipoprotein particles is severely impaired [4] (S.G. Young, 1993). In cultured rat hepatoma cells transfected with cDNAs encoding C-terminally truncated variants of human apo B100 (from apo B15 to B94) the size and density of the secreted apo B-containing particles are dependent upon the length of the apo B. For example, apo B18 is secreted essentially without neutral lipids whereas apo B28 is secreted in particles the size and density of high-density lipoproteins. As the size of the apo B molecule increases, from B37 to B48 to B53 to B72 to B94, the apo B associates with progressively more lipid in larger and less dense particles [5]. Thus, the sequence of apo B between apo B17 and B41 is necessary for assembly of TG-rich lipoproteins. Sequences in the C-terminus of apo B29 bind phospholipids and diacylglycerols but only a little TG. TGs, however, associate with the region between apo B29 and B32.5, and a massive accumulation of TGs occurs between B32.5 and B41 where ~ 1 molecule of TG binds for each 2 amino acids of apo B (D.M. Small, 2000).

The four alternating α -helices and β -sheets (Fig. 3) are common features of apo B100 in all vertebrate species examined. Amphipathic β -strands are also found in the egg yolk protein, lipovitellin. The X-ray crystal structure of lamprey lipovitellin reveals a 'lipid pocket' joined by three anti-parallel β -sheets. Based on the prediction that human apo B100 contains a similar lipid-binding pocket at the N-terminal domain of the molecule, a model has been proposed in which this pocket provides the initial site of association of TGs with apo B. Sequential accumulation of TGs within this cavity, in concert with expansion of the pocket, is thought to occur by unfolding of the amphipathic β -strands of the $\beta 1$ -domain (J. Segrest, 1999). Not explained by these studies or models, however, is the reason why apo B48 assembles into chylomicrons, the largest and most TG-rich particles, whereas apo B100, which is approximately twice as large as apo B48, forms the smaller VLDLs.

3. *Transcriptional regulation of apo B synthesis*

3.1. *DNA elements regulating apo B transcription*

Under most metabolic conditions, changes in apo B mRNA levels do not influence how much apo B is secreted. Moreover, changes in apo B secretion, over at least a 7-fold range, occur without alteration in the amount of apo B mRNA (J. Scott, 1989). Consequently, it is generally accepted that apo B secretion is primarily regulated co- and post-translationally.

The liver and intestine produce the majority of apo B, but in mice the production of apo B in the yolk sac is crucial during fetal development. It is thought that apo B plays a role in transferring lipid nutrients to the developing mouse embryo (R.V. Farese, 1999).

Interestingly, apo B is also expressed at low levels in the hearts of humans and mice (S.G. Young, 1998). The presence of apo B and microsomal triacylglycerol transfer protein (which is also required for lipoprotein assembly, Section 6.4) in the heart presumably leads to the secretion of apo B100-containing lipoproteins by cardiac myocytes. Recent experiments suggest that secretion of TG-rich lipoproteins by the heart is a mechanism for regulating the amount of TGs stored in the heart and thereby protects the heart from a detrimental accumulation of lipids [6].

The human apo B gene spans 43 kb, is located on chromosome 2p and consists of 28 introns and 29 exons. Over half of the apo B coding sequence is encoded by one exceptionally large exon, exon 26, which contains 7572 bp. The apo B mRNA transcript is 14 kb and is remarkably stable, having a half-life of 16 h (J. Scott, 1989). Some elements responsible for expression of the human apo B gene have been defined. The gene promoter (bp -898 to +1) contains a TATA box. Within the promoter region are sequences that bind the liver-specific transcription factors HNF-3 and NF-1. Positive and negative *cis*-acting DNA sequences required for transcription of apo B in the liver were identified using transient transfection assays of promoter-reporter constructs in cultured human hepatoma (HepG2) cells and intestinal (CaCo2) cells. The majority of enhancer activity was localized to 443 bp within intron 2 [7]. By footprint analysis this enhancer region was shown to bind two nuclear transcription factors, HNF-1 and C/EBP. An additional, weaker, enhancer was found within intron 3, and a reducer element was identified in the 5'-flanking region between bp -3211 and -1802. However, when these promoter elements were linked to reporter genes and expressed in transgenic mice, a more complex picture emerged. In mice, both the proximal promoter (bp -898 to +1) and intron 2 enhancer were required for expression of a β -galactosidase reporter in liver, yet surprisingly no intestinal expression occurred even though the same constructs induced expression in the intestinal cell line, CaCo2. Moreover, the reducer element (bp -3200 to -1802) that was identified in transient transfection studies in HepG2 cells, did not decrease expression in livers of transgenic mice. Consequently, while CaCo2 and HepG2 cells are frequently used as experimental models of the intestine and liver, respectively, it is important to verify in intact animals results obtained from cultured cells.

Recently, factors controlling the intestinal expression of apo B have been described.

A high level of intestinal expression of human apo B in transgenic mice was achieved with a construct containing ~80 kb of the 5'-flanking sequence [8]. The region of the gene driving expression of apo B transgenes in mouse intestine was subsequently localized to a 3 kb segment over 55 kb upstream of the transcriptional start site [9,10]. This region contains putative binding sites for the transcription factors HNF-3 β , HNF-4 and C/EBP β ; the corresponding segment of the murine apo B gene exhibits a high degree of sequence conservation. Interestingly, elements of the apo B gene that had been previously demonstrated *in vivo* to confer expression in the liver (i.e. intron 2 enhancer and 5' upstream liver enhancer) do not play a role in intestinal expression of apo B transgenes in mice.

3.2. Apo B mRNA editing

Apo B100 contains 4536 amino acid residues and in humans is the only form of apo B produced by the liver. In contrast, the small intestine of all mammals, and the liver of some species, synthesize apo B48, the N-terminal 2152 residues of apo B100. Apo B100 and apo B48 are generated from a single structural gene. Subsequently, apo B48 is produced by an unusual RNA editing process in which a single cytidine at position 6666 of human apo B mRNA is deaminated to a uridine [11]. A stop codon is thereby introduced and the truncated variant, apo B48, is generated. The *cis*-acting elements directing this site-specific deamination are located within approximately 25 nucleotides in an AU-rich region flanking the target cytidine. A region of 11 nucleotides 5 bases downstream of this cytidine, is particularly important for editing and has been called the 'mooring sequence'.

A 27 kDa protein from enterocytes is required for apo B RNA editing [12]. This protein, called apobec-1 (for 'apo B editing complex-1'), is highly homologous to other cytidine deaminases but does not, by itself, edit the RNA. Rather, apobec-1 is the catalytic subunit of a multi-protein complex containing auxiliary proteins. Apobec-1 is a homodimer that is present in both the cytosol and nucleus and apo B48 is formed only in tissues that express apobec-1 mRNA (i.e. human and rodent intestine and rodent liver). However, apobec-1 is also expressed in some tissues that do not make apo B, suggesting that this enzyme also acts on other mRNAs in addition to apo B mRNA. This idea is supported by the finding that transgenic mice over-expressing apobec-1 develop hepatocellular carcinomas and liver dysplasia (T.L. Innerarity, 1995).

Apo B48-containing lipoproteins are removed from plasma more rapidly than apo B100-containing lipoproteins since apo E, which is added to apo B48-containing lipoproteins after secretion, is a high-affinity ligand for the LDL receptor (Chapter 21). It was reasoned, therefore, that if apo B100 secretion were blocked, by inducing the complete editing of apo B mRNA so that only apo B48 was produced, fewer circulating apo B-containing lipoproteins would be present and, consequently, the animals would be less susceptible than normal to diet-induced hypercholesterolemia. As predicted, when the apobec-1 gene was over-expressed in livers of atherosclerosis-prone mice via adenovirus-mediated gene transfer, plasma apo B100-containing lipoproteins and cholesterol were greatly decreased and the extent of atherosclerosis was reduced (B. Teng, 1994).

On the other hand, targeted disruption of the murine apobec-1 gene abolished apo B editing in all tissues and resulted in a complete deficiency of apo B48 in serum (N.O. Davidson, 1996) demonstrating that there is no functional duplication of the apobec-1 gene. Apobec-1^{-/-} mice are fertile, healthy, and serum levels of cholesterol and TG are normal showing that the apobec-1 gene is not essential for normal life. Moreover, mice that express only apo B48 (by replacement of the apo B48-editing codon with a stop codon) show no obvious defects in growth, reproduction or function (S.G. Young, 1996). A question that remains is: why is apo B100 produced at all?

4. Models used for studying apo B and VLDL secretion

The most commonly used models for studying the assembly and secretion of apo B-containing lipoproteins are hepatoma cell lines (HepG2 human hepatoma cells and McArdle 7777 rat hepatoma cells) and primary hepatocytes from rats, mice or hamsters. Each model has its limitations. In addition, several transgenic and gene-targeted mouse models have been developed.

Hepatoma cell lines are convenient laboratory models since they are easily maintained and manipulated under defined conditions. However, although hepatoma cells have retained many properties of hepatocytes, several key liver functions, including some important aspects of lipid metabolism, have been altered. One advantage of HepG2 cells is that they are of human origin and, like human liver, secrete apo B100 but not apo B48. However, the basal rate of apo B secretion in HepG2 cells is low, and the apo B-containing lipoproteins are not true VLDLs but contain less lipid than VLDLs. Thus, the use of HepG2 cells for studying VLDL assembly is not ideal. McArdle 7777 hepatoma cells are of rat origin and, like rat liver, secrete both apo B100 and B48. When incubated with oleate some of the secreted apo B is in VLDLs, but the majority of apo B is secreted in more dense, partially lipidated, lipoproteins. One advantage of McArdle cells is that they are excellent cells in which to express heterologous cDNAs, such as those encoding truncated and mutated forms of apo B.

Primary hepatocytes isolated from livers of rats, mice and hamsters are also commonly used for studying VLDL assembly and secretion. Although these cells must be freshly isolated for each experiment, they retain most properties of native hepatocytes, at least for 24 h. Unlike human hepatocytes, primary rat and mouse hepatocytes secrete both apo B48 and apo B100 but these particles are the size, composition and density of VLDLs. Hamster primary hepatocytes are also useful since they secrete VLDLs that contain apo B100, but not apo B48 (K. Adeli, 2000). However, stable transfection of primary hepatocytes with cDNAs is not possible.

Several gene-targeted mice have been effectively used to study apo B metabolism [4]. Expression of human apo B in mice fed a high-fat diet resulted in a 20-fold increase in the plasma content of apo B-containing lipoproteins and extensive atherosclerosis. Since the secretion of apo B from hepatocytes of these mice was only slightly higher than in wild-type mice the lipoprotein accumulation and atherosclerosis were attributed primarily to defective lipoprotein clearance. Another line of transgenic mice was produced in which Arg-3500 of apo B (the proposed site of binding to the LDL

receptor) was mutated. These mice were unable to clear apo B-containing lipoproteins from the plasma and developed severe hypercholesterolemia.

Generation of apo B knock-out mice was problematic since most apo B^{-/-} embryos died during development. Nevertheless, mice lacking the murine apo B gene, but expressing human apo B, were generated. During the suckling period the offspring were indistinguishable from wild-type mice but because the human apo B gene was not expressed in the intestine (Section 3.1) the mice did not produce chylomicrons and suffered severe fat malabsorption and retarded growth [8].

The disease hypobetalipoproteinemia is caused by point mutations in the apo B gene that result in production of low levels of poorly lipidated, truncated apos B. Several mouse models of this disorder have been generated. For example, transgenic mice expressing apos B70, B81 or B83 have low levels of plasma apo B-containing lipoproteins and develop severe neurodevelopmental abnormalities [4].

5. Covalent modifications of apo B

Several co- and post-translational modifications of apo B occur. Human apo B100 contains 25 cysteine residues of which 16 exist as intramolecular disulfide bonds; 14 of these are clustered in the N-terminal region. Disulfide bond formation likely occurs co-translationally by the action of protein disulfide isomerase, a luminal ER protein that coordinates disulfide bond formation and protein folding. As discussed in Section 6.4, protein disulfide isomerase is one subunit of the microsomal TG transfer protein heterodimer that is required for efficient secretion of apo B-containing lipoproteins. To determine if disulfide bond formation in the N-terminus of apo B were required for assembly of lipid with apo B, individual cysteine residues were replaced with serines or alanines. When either or two cysteine pairs in this region was eliminated, lipid assembly and secretion of apo B were greatly reduced (G.S. Shelness, 1997; Z. Yao, 1998) implying that disulfide bonds in this region are critical for the correct folding and secretion of apo B.

Plasma apo B100 contains 8–10% by weight carbohydrate and at least 20 potential *N*-linked glycosylation sites. Both *N*- and *O*-linked glycosylations occur in apo B100. *N*-linked glycosylation begins co-translationally in the ER lumen but it is not yet clear whether or not glycosylation is required for apo B secretion. Treatment of chicken hepatocytes with tunicamycin, which inhibits formation of *N*-linked oligosaccharide chains, did not reduce the amount of apo B secreted (D.M. Lane, 1982). However, when HepG2 cells were incubated with tunicamycin, apo B100 secretion was reduced and the intracellular degradation of apo B (Section 7) was increased. Moreover, the apo B100-containing particles that were secreted had a normal buoyant density suggesting that *N*-linked glycosylation is not required for assembly of lipids with apo B100 (L. Chan, 2001). An alternative explanation, however, is that the decreased secretion of apo B100 resulted from inadequate lipidation of apo B so that defective particles underwent pre-secretory degradation.

Several studies have shown that secreted apo B contains multiple phosphorylated serine residues (R.A. Davis, 1984) and that the phosphorylation is increased in diabetic,

compared to non-diabetic, rats (J.D. Sparks, 1990). The physiological relevance of this phosphorylation is not known. Apo B phosphorylation has been suggested to occur in the Golgi apparatus in agreement with the finding that the Golgi contains kinases that phosphorylate secretory proteins (L.L. Swift, 1996).

Human apo B secreted by HepG2 cells is also covalently acylated with palmitic acid on at least one cysteine residue (J.M. Hoeg, 1988). Mutation of Cys-1085 in human apo B29 expressed in McArdle hepatoma cells abolished palmitoylation at this site. Interestingly, the secreted lipoproteins containing the mutated apo B29 were associated with less TG than those containing apo B29 with palmitoylated Cys-1085 [13]. These studies suggest that palmitoylation of apo B might play a role in assembling neutral lipids with apo B. However, no information is available on how many cysteines of apo B are palmitoylated, which palmitoyltransferase is involved or the topology of the palmitoylation reaction.

6. Regulation of apo B secretion by lipid supply

6.1. Fatty acids and triacylglycerols

In most instances, apo B is synthesized constitutively and in excess of the amount secreted so that the rate of apo B synthesis does not regulate how much apo B is secreted. The assembly of apo B-containing lipoproteins requires the synthesis of apo B, TGs, cholesterol, cholesteryl esters and phospholipids. When insufficient lipid is available for assembly of apo B into stable lipoprotein particles, excess apo B is not secreted but is degraded intracellularly, primarily by the proteasome and also by undefined proteases within the lumen of the secretory pathway (Section 7).

When the fatty acid oleate is added to HepG2 cells the synthesis of TG and phospholipids is stimulated and the amounts of apo B and TG secreted are increased [14]. Thus, an increased lipid supply enables a larger proportion of newly synthesized apo B to be translocated across the ER membrane and enter the secretory pathway. In agreement with the idea that lipid supply can regulate apo B secretion, the oleic-acid-induced increase in apo B secretion is blocked by Triacsin D, an inhibitor of TG synthesis. The rate of apo B secretion is not, however, solely a function of the rate of TG synthesis since glucose stimulates both TG synthesis and secretion, but does not increase apo B secretion. Furthermore, when oleic acid is supplied to primary rat hepatocytes, in contrast to HepG2 cells, both the synthesis and secretion of TG are increased whereas apo B secretion is unaltered. Therefore, the difference in response of apo B secretion to oleate in HepG2 cells versus primary hepatocytes cannot be ascribed to differences in TG synthesis.

Early work suggested that two pools of TG exist in hepatocytes: a large cytosolic storage pool that turns over slowly, and a small microsomal pool that turns over rapidly. The source of TG used for assembly with apo B appears to be primarily (perhaps ~70%) the cytosolic storage pool rather than the pool made from de novo synthesis [15]. Recent data support a model in which cytosolic TG is hydrolyzed, perhaps by the recently discovered TG hydrolase in the ER lumen [16]. The mechanism by which

an ER luminal lipase accesses cytosolic TG is not clear but the small amount of TG (~5% of total membrane lipids) in the ER membrane is a potential source of this TG. The lipolysis products (diacylglycerols and monoacylglycerols) are thought to be subsequently re-esterified within the ER lumen to produce TG that is assembled with apo B.

Operation of this lipolysis–re-esterification cycle also requires fatty acids, as well as a mono- or di-acylglycerol acyltransferase activity, in the ER lumen for re-synthesis of TG. Until recently, all TG was thought to be synthesized by diacylglycerol acyltransferase-1, a microsomal enzyme (Chapter 10). Surprisingly, however, diacylglycerol acyltransferase-1 knock-out mice retain the ability to synthesize TG and have normal fasting serum TG levels [17]. These data indicate that secretion of TG-rich lipoproteins does not require diacylglycerol acyltransferase-1. Consequently, another gene product has been implicated in synthesizing TG for VLDL assembly: diacylglycerol acyltransferase-2 [18]. Consistent with this idea, diacylglycerol acyltransferase-2 is highly expressed in liver. Confirmation of this hypothesis awaits generation of diacylglycerol acyltransferase-2-deficient mice.

The type of fatty acid supplied to hepatocytes also influences the secretion of apo B-containing lipoproteins. Compared to oleic acid, the (n-3) fatty acids eicosapentaenoic acid (20:5) and docosahexaenoic acid (22:6), found in fish oils, decrease plasma TG levels in humans and decrease the secretion of apo B-containing lipoproteins from rat hepatocytes and hepatoma cells. Consistent with these findings, (n-3) fatty acids also stimulate apo B degradation (E.A. Fisher, 1993).

6.2. Phospholipids

Phosphatidylcholine is the major phospholipid on the surface monolayer of all lipoproteins including VLDLs. In the liver, phosphatidylcholine is synthesized by two biosynthetic pathways: the CDP-choline pathway and the phosphatidylethanolamine *N*-methylation pathway (Chapter 8). Choline is an essential precursor of phosphatidylcholine synthesis via the CDP-choline pathway. When cells or animals are deprived of choline, this biosynthetic pathway is specifically inhibited and secretion of TGs and apo B, but not other proteins, is reduced by ~70% even though the phosphatidylcholine content of ER and Golgi membranes is essentially normal [19]. Thus, phosphatidylcholine synthesis is required for VLDL secretion. Interestingly, choline deficiency inhibits the secretion of only apo B variants larger than apo B28 (i.e. only those that assemble a neutral lipid core) indicating that association of apo B with neutral lipids and/or VLDL secretion depend on phosphatidylcholine synthesis. The mechanism by which decreased synthesis of phosphatidylcholine inhibits apo B secretion is by pre-secretory degradation. Translocation of apo B into the ER lumen is not impaired since the number of apo B-containing particles within the ER lumen is normal. In contrast, the number of apo B-containing particles in the Golgi lumen is decreased by ~70% implying that defective VLDL particles are degraded in a post-ER compartment of the secretory route (Section 7).

An alternative pathway for phosphatidylcholine synthesis, which is quantitatively significant only in the liver, is the methylation of phosphatidylethanolamine to phos-

phatidylcholine via phosphatidylethanolamine *N*-methyltransferase. Male mice in which the methyltransferase gene was disrupted (D.E. Vance, 1997) have greatly reduced plasma levels of TG and apo B100, but not apo B48. Moreover, the secretion of apo B100 and TG from hepatocytes derived from these mice is similarly impaired (A. Noga, 2002). Thus, phosphatidylcholine synthesis is required for normal apo B secretion.

Phospholipid turnover also appears to play a role in VLDL assembly (G.F. Gibbons, 1996). A significant proportion of TG in VLDLs contains fatty acids derived from the deacylation of phospholipids. Moreover, the assembly of VLDLs has been proposed to require the calcium-independent phospholipase A2 (Z. Yao, 2000).

In addition to phosphatidylcholine, VLDLs contain smaller amounts of other phospholipids (e.g. phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol) as well as sphingomyelin and ceramide. Inhibition of sphingolipid synthesis by >90% by addition of fumonisin B to rat hepatocytes (Chapter 14), does not, however, inhibit VLDL secretion. Therefore, normal amounts of sphingolipids are not required for VLDL assembly/secretion (A.H. Merrill, 1995).

6.3. Cholesterol and cholesteryl esters

Sterol response element binding proteins (SREBPs) are master regulators of fatty acid and cholesterol synthesis at the level of transcription (Chapters 6, 7, 15). The transcriptionally active forms of SREBPs are produced from precursor proteins by a sterol-dependent proteolytic cleavage [20]. SREBPs co-ordinately increase the expression of genes involved in synthesizing fatty acids (fatty acid synthase, acetyl-CoA carboxylase, stearoyl-CoA desaturase) and cholesterol (3-hydroxy-3-methylglutaryl-CoA reductase), as well as the microsomal TG transfer protein (Section 6.4). All these components are required for VLDL assembly. The ability of the liver to secrete VLDLs is also linked via SREBP to the cholesterol/bile acid pathway through changes in the hepatic level and metabolism of cholesterol (R.A. Davis, 2001).

Cholesteryl esters are relatively minor constituents (5–15% of total lipids) of VLDLs but the amount of cholesteryl esters relative to TGs increases when rats are fed a high-cholesterol diet (R.A. Davis, 1982). There are conflicting data on whether or not the availability of cholesterol and/or cholesteryl esters directly influences apo B secretion. Inhibition of cholesteryl ester formation in hepatocytes decreases apo B secretion in some studies but not others. A severe reduction in the cholesteryl ester content of hepatoma cells reduces apo B secretion whereas an increased cellular content of cholesteryl esters above normal does not stimulate apo B secretion. Cholesterol is esterified by acyl-CoA:cholesterol acyltransferase (ACAT) which is encoded by at least two different genes. In mouse liver and intestine the majority of cholesteryl esters are made via ACAT-2. In animal studies, ACAT inhibitors reduce plasma cholesterol probably primarily by decreasing intestinal cholesterol absorption rather than by reducing VLDL secretion. In support of this idea, targeted disruption of the ACAT-2 gene in mice greatly reduces intestinal cholesterol absorption and results in complete resistance to diet-induced hypercholesterolemia (R.V. Farese, 2000). However, apo B-containing lipoproteins are made in normal amounts in ACAT-2^{-/-} mice despite

the absence of essentially all hepatic ACAT activity. Interestingly, plasma VLDLs of these mice have smaller diameters than those of wild-type mice. Thus, normal amounts of cholesteryl esters might be required for assembling large VLDLs. In mice with a targeted disruption of the ACAT-1 gene, hepatic cholesterol esterification activity and plasma cholesterol levels are normal [21].

Based on experiments with ACAT^{-/-} mice it has been suggested that ACAT-1 functions primarily in providing cholesteryl esters for storage in cytosolic droplets whereas ACAT-2 is linked to secretion of cholesteryl esters into lipoproteins. In agreement with this concept, the two ACATs have been proposed to have different membrane topologies with the active site of ACAT-1 facing the cytosol and that of ACAT-2 facing the ER lumen (L. Rudel, 2000). It is not yet clear, however, how these findings apply to humans since ACAT-1 accounts for the majority of ACAT activity in *human* hepatocytes whereas ACAT-2 is the major ACAT in human intestine.

6.4. Microsomal triacylglycerol transfer protein

Lipids are assembled with apo B into VLDLs in the ER lumen (J.E. Vance, 1993). During this process TGs are concentrated into the core of the particle. A microsomal luminal protein, the 97 kDa microsomal triacylglycerol transfer protein (MTP), that has the ability to transfer TGs between membranes *in vitro*, is proposed to transfer TG to nascent apo B particles [22]. MTP is present in liver and intestine as a soluble heterodimer with protein disulfide isomerase (55 kDa) (J.R. Wetterau, 1990). The latter is a ubiquitous ER luminal protein that catalyzes formation of disulfide bonds during protein folding. The 97 kDa MTP subunit confers all lipid transfer activity to the heterodimer. In *in vitro* assays, MTP catalyzes lipid transfer from donor to acceptor liposomes with substrate specificity of: TG > cholesteryl esters > diacylglycerols > phosphatidylcholine. The lipid transfer reaction displays ping-pong, bi-bi kinetics implying that MTP transfers lipids by a 'shuttle' mechanism. Although MTP contains no C-terminal KDEL sequence (this sequence retains soluble proteins within the ER lumen), association of MTP with protein disulfide isomerase, which does contain a KDEL sequence, is thought to retain MTP within the ER lumen. The tissue and subcellular location of MTP, and its preference for transporting neutral lipids, suggest that MTP is involved in loading nascent apo B particles with TG.

6.4.1. MTP deficiency in humans and mice

The hypothesis that MTP is required for secretion of apo B-containing lipoproteins from the liver and intestine was strengthened by the discovery that individuals with the rare genetic disease abetalipoproteinemia lack detectable MTP protein and intestinal MTP lipid transfer activity (J.R. Wetterau, 1992). These patients suffer from fat malabsorption and despite a normal apo B gene, their plasma apo B is barely detectable. Specific inhibitors of MTP lipid transfer activity have been developed based on the premise that MTP might be a target for reducing atherosclerosis by inhibiting production of apo B-containing lipoproteins. MTP inhibitors lower plasma cholesterol levels by up to 80% in rats, hamsters and rabbits (J.R. Wetterau, 1998). However, heterozygosity for MTP deficiency in humans does not result in altered plasma lipid or lipoprotein levels

suggesting that MTP is not normally rate-limiting for lipoprotein production unless MTP activity is greatly reduced.

The function of MTP has been investigated in genetically modified mice [23,24]. Complete elimination of the murine MTP gene is embryonically lethal, probably because apo B-containing lipoproteins are required for transferring lipids from the yolk sac to the developing mouse embryo. However, the murine MTP gene was inactivated specifically in the liver by generating mice harboring a 'floxed' MTP gene and subsequent Cre-mediated recombination. In these mice, plasma apo B100 levels were reduced by >90% but, surprisingly, apo B48 levels were reduced only slightly. Moreover, in hepatocytes derived from these mice apo B100 secretion was eliminated whereas secretion of apo B48 was almost unaltered. However, the apo B48-containing lipoproteins contained less lipid than those from wild-type mice. In addition, ultrastructural analyses revealed that, compared to wild-type hepatocytes, MTP^{-/-} hepatocytes contained very few VLDL-sized, lipid-staining particles within the ER or Golgi lumina. Moreover, MTP^{-/-} mouse livers contained numerous lipid droplets in the cytosol, a hallmark of impaired TG secretion. These observations suggest that (i) MTP is required for transferring TG into the lumen of the ER, and (ii) apo B100 secretion requires MTP whereas secretion of apo B48 is either less sensitive to MTP deficiency or does not require MTP. On the other hand, over-expression of MTP in mouse liver via recombinant adenoviruses increased the secretion and plasma levels of apo B100 and B48, as well as TG (D.J. Rader, 1999).

It should be noted that although MTP is assumed to function as a lipid transfer protein, based on its ability to transfer lipids between membranes *in vitro*, a lipid transfer function of MTP in providing lipid for VLDL assembly has not been directly demonstrated in intact cells or animals.

6.4.2. *Studies on the function of MTP in cultured cells*

Recently, investigators have extensively studied the role of MTP in the assembly of apo B-containing lipoproteins in hepatocyte-derived cells, as well as non-hepatic cells that have been genetically modified to express apo B and MTP. MTP and apo B have been shown by co-immunoprecipitation experiments to interact physically during lipoprotein assembly via specific sites that have been identified on MTP and apo B (C.C. Shoulders, 1999; G.S. Shelness, 1998; T. Grand-Perret, 1999). The association between MTP and apo B is transient and increases when lipoprotein assembly is stimulated upon addition of oleic acid, and decreased when TG synthesis is inhibited by Triacsin D (H.N. Ginsberg, 1996). Apo B also co-immunoprecipitates with other ER luminal chaperone proteins such as calnexin, calreticulin, GRP94, Erp72 and BiP which presumably facilitate the correct folding of apo B (H. Herscovitz, 1998). MTP itself has also been implicated as a chaperone for apo B during VLDL assembly (H.N. Ginsberg, 2001).

There is considerable controversy regarding the precise role of MTP in assembling lipids with apo B [25]. MTP has been suggested to play a role in several steps along the VLDL assembly pathway: translocation of apo B across the ER membrane and into the ER lumen; co-translational assembly of lipids with apo B; transfer of the bulk of TG into the core of VLDLs; movement of TG into the ER lumen. Experimental data both support and refute the participation of MTP at each of these steps. Some of the conflicting data likely arise from different experimental models used, e.g. hepatoma

cells versus primary hepatocytes, cultured cells versus genetically modified mice, human versus rodent models.

A general consensus is that lipid addition to apo B begins during translation and translocation of apo B across the ER membrane (E.A. Fisher, 1998; D.A. Gordon, 1996; S.-O. Olofsson, 1998). Experiments with cultured cells indicate that MTP stimulates, but is not absolutely required for, co-translational lipidation of apo B. Short apo B variants, such as apo B18, are secreted from hepatoma cells in poorly lipidated form independent of MTP, whereas secretion of longer (i.e. >apo B23), more highly lipidated apo B variants is stimulated by MTP. These findings suggest that the TG-transferring property of MTP is important for VLDL assembly. Moreover, the requirement of MTP is much greater for the secretion of apo B100 than for apo B48 suggesting that there might be different mechanisms involved in assembly of these two types of lipoproteins. In spite of a suggested chaperone function for MTP, this protein is apparently not *required* for translocation of apo B across the ER membrane since (i) apo B41-containing lipoproteins are secreted from mammary-derived cells lacking MTP activity (D.M. Small, 1995), (ii) apo B48 is lipidated and translocated equally well into the lumen of dog pancreatic microsomes (which lack MTP) and liver microsomes (which contain MTP) (A.E. Rusñol, 1997), and (iii) an almost normal amount of apo B48, albeit poorly lipidated, is secreted from MTP^{-/-} hepatocytes (S.G. Young, 1998).

Whether or not MTP plays a role in supplying the bulk of TG for VLDLs is controversial. Several studies indicate that MTP is not directly involved in this process. One suggestion is that MTP transports TGs into the ER lumen to form luminal TG droplets which are subsequently incorporated into apo B-containing lipoproteins. This proposed role for MTP is derived from observations that (i) MTP^{-/-} livers of mice lack VLDL-sized, lipid-staining particles that are normally present in the ER lumen of wild-type mice, and (ii) ER luminal lipid droplets, the size of chylomicrons, accumulate in enterocytes of mice lacking intestinal apo B (R.L. Hamilton, 1998). However, a role for MTP in transferring TG to apo B, either by shuttling TG monomers to apo B or by mediating the fusion between partially lipidated apo B particles and a luminal TG droplet, has not been unequivocally demonstrated. Nor is it clear how the proposed 'shuttle mechanism' for MTP can be reconciled with a role in transferring such large amounts of TG to apo B.

Based on available experimental evidence several models have been proposed for the assembly of apo B with lipids. In one popular model, called the 'two-step' model (Fig. 4A), MTP transfers lipid to apo B during translocation of the protein into the ER lumen to form partially lipidated apo B. These small particles then fuse with a TG droplet in the ER lumen to produce VLDLs. As discussed above, MTP might be involved in forming the luminal TG droplet and/or in fusion of the droplet with the small apo B-containing particle. However, the biophysical mechanism by which a TG droplet would fuse with a small apo B-containing lipoprotein is not clear. A second model of assembly of apo B-containing lipoproteins is one in which lipid is sequentially added to apo B that is either undergoing translocation across the membrane, or that is associated with the luminal face of the ER membrane (Fig. 4B). During times when lipid supply is low, small, lipid-poor apo B particles would be formed and secreted, but when lipid supply is abundant, fully lipidated VLDLs would be produced.

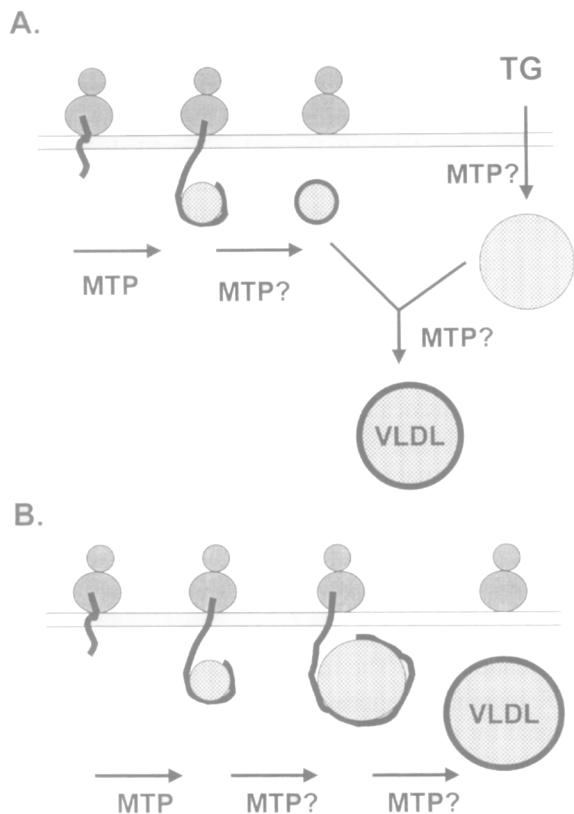


Fig. 4. Two models proposed for assembly of apo B-containing lipoproteins in hepatocytes. (Panel A) The 'two-step' model. Shown from left to right is the co-translational translocation of apo B into the ER lumen during which small amounts of lipid are assembled with apo B in a MTP-dependent step. The partially lipidated apo B particle is then released into the lumen and fuses with a luminal triacylglycerol (TG) droplet to form VLDL. Alternatively, MTP might 'shuttle' TG monomers to the particle. It is proposed that MTP mediates formation of a TG droplet in the ER lumen for assembly with apo B. (Panel B) The concerted assembly model. From left to right is shown the co-translational translocation of apo B into the ER lumen. Lipid is sequentially added to apo B during translocation until a VLDL-sized particle is formed. In the event of limited lipid supply, small, partially lipidated apo B particles would be secreted. The bulk of TG might be transferred either from TG within the ER membrane or from a luminal TG droplet, as indicated in panel A. The role of MTP in some steps of both schemes (indicated by ?) is still controversial.

Many issues remain concerning the role of MTP in the assembly of apo B-containing lipoproteins. (i) The existence of ER luminal TG droplets has not been unambiguously demonstrated by isolation of these droplets from hepatocytes. (ii) The mechanism of formation and stabilization of TG droplets in the ER lumen is not understood. (iii) It is not clear why some luminal TG droplets lacking apo B would not be secreted by default from hepatocytes. (iv) The partially lipidated apo B-containing particles that are formed co-translationally have not been directly shown to be precursors of fully lipidated VLDLs. (v) It is not clear why MTP deficiency abrogates the secretion of apo B100 but not apo B48.

6.4.3. MTP gene expression

The promoter region of the MTP gene contains elements predicted to bind transcription factors (e.g. HNF-1, HNF-4 and AP-1) that might dictate the cell type-specific expression of MTP. Hepatic MTP mRNA levels are increased in hamsters fed a high-fat diet. The activity of the human promoter is increased by cholesterol, most likely via the SREBP pathway (Chapter 15), and expression of the MTP gene is inhibited by insulin. However, it is not yet known if these alterations in gene expression regulate the secretion of apo B-containing lipoproteins.

7. Translocation and intracellular degradation of apo B

Typical secretory proteins co-translationally translocate across the ER membrane without interacting with the membrane bilayer or being exposed to the cytosol. However, translocation of apo B into the ER lumen is unusual in several respects. First, complete translocation of apo B occurs only when apo B is associated with lipid. Second, an unusual translocational pausing occurs during which translation continues and domains of newly synthesized apo B become exposed to the cytosol (V.R. Lingappa, 1990). Translocational pausing of apo B has been proposed to be mediated by multiple 'pause-transfer sequences' which are similar, but non-identical, sequences of ~10 amino acids that direct stopping and starting during translocation [26]. The translocating chain-associated membrane protein, TRAM, in the ER membrane appears to mediate translocational pausing (V.R. Lingappa, 1998). The signal for resumption of translocation after pausing is not known but has been suggested to be association of lipid with apo B. Others have argued that translational, rather than translocational, pausing occurs.

7.1. Proteasomal degradation of apo B

A third unusual property of apo B is that its translocation across the ER membrane is linked to proteasomal degradation (Fig. 5A). Pulse-chase studies in hepatocytes show that 40 to 60% of newly synthesized apo B is not secreted but is degraded intracellularly [27]. When lipid supply is restricted, or when MTP is inhibited, apo B does not efficiently translocate into the ER lumen but becomes exposed to the cytosol and is degraded. However, this degradation does not determine how much apo B is secreted because when degradation is inhibited by *N*-acetyl-Leu-Leu-norleucinal, apo B accumulates in microsomes whereas apo B secretion is not increased (R.A. Davis, 1994). This arrest in translocation and cytosolic exposure of apo B result in apo B being ubiquitinated and targeted for degradation by cytosolic proteasomes [28] in a process facilitated by the cytosolic chaperone hsp70 (E.A. Fisher, 2001). Several ER luminal and membrane proteins are known to undergo proteasomal degradation by a pathway in which the completely translocated protein undergoes retrotranslocation into the cytosol and is subsequently ubiquitinated and degraded by proteasomes. In contrast, the ubiquitination and proteasomal degradation of apo B are thought to be initiated while apo B is attached to the ribosome and still within the translocation channel (E.A. Fisher, 2001).

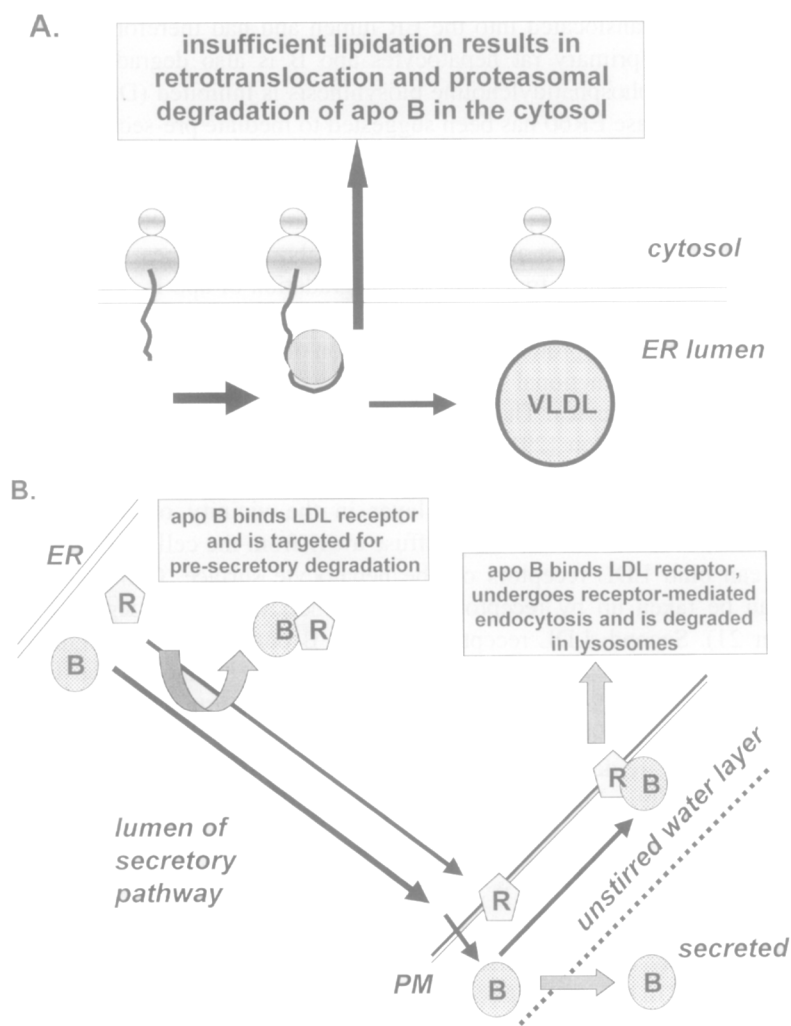


Fig. 5. Intracellular degradation of apo B. (Panel A) When lipid supply is limited, or when MTP is inhibited, lipid-poor apo B particles are formed and apo B is exposed to the cytosol, becomes ubiquitinated and degraded by the proteasome. Sufficient supply of lipids results in VLDL being secreted. (Panel B) Apo B is also degraded non-proteasomally. Nascent apo B particles, indicated as 'B' are transported via the secretory route to, and across, the plasma membrane (PM) and enter the 'unstirred water layer' adjacent to the cell. Some particles are fully secreted whereas others bind to cell surface LDL receptors, indicated by 'R', are internalized by receptor-mediated endocytosis and undergo lysosomal degradation. The LDL receptor is also proposed to mediate the pre-secretory degradation of apo B by binding to apo B100 within the lumen of the secretory pathway and targeting apo B100 for degradation by as yet unidentified luminal proteases.

7.2. Degradation of apo B within the secretory pathway

Apo B is also degraded within the secretory pathway. This site of degradation was demonstrated in HepG2 cells in which apo B was degraded by a luminal protease after

the protein had completely translocated into the ER lumen and had therefore escaped proteasomal degradation. In primary rat hepatocytes apo B is also degraded within the secretory pathway when phosphatidylcholine biosynthesis is inhibited (D.E. Vance, 1993). The ER luminal protease ER60 has been suggested to mediate pre-secretory apo B degradation (K. Adeli, 1997). Degradation of apo B within the secretory pathway is thought to provide a quality control mechanism for eliminating defective particles before their secretion. In contrast to the proteasomal degradation of apo B, which occurs in *response* to inefficient translocation across the ER membrane but does not control how much apo B is secreted, the post-translocational degradation of apo B can directly control how much apo B is secreted.

A clue to the mechanism of non-proteasomal degradation of apo B came from studies in which hepatocytes lacking functional LDL receptors were found to secrete more apo B than did wild-type hepatocytes. The LDL receptor was subsequently implicated in two facets of apo B degradation [29] (Fig. 5B). First, when fully assembled apo B-containing particles are exported across the plasma membrane of hepatocytes, the nascent lipoproteins enter the unstirred water layer in the vicinity of the plasma membrane. These particles do not immediately diffuse away from the cell (E.A. Fisher, 1990) but instead can bind LDL receptors on the hepatocyte surface. Consequently, the lipoproteins can be taken up by receptor-mediated endocytosis and degraded in lysosomes (Chapter 21). Second, LDL receptors and apo B both transit the secretory pathway en route to the plasma membrane. The ligand-binding domain of the LDL receptor is oriented towards the ER lumen and therefore has the opportunity to bind apo B intralumenally and target it for degradation before it can be secreted. Approximately equal amounts of apo B100 are degraded via the LDL receptor using the re-uptake and pre-secretory mechanisms [29]. Apo B100 is more susceptible than apo B48 to LDL receptor-mediated degradation because apo B100 contains the LDL receptor-binding domain that is absent from apo B48. Small amounts of apo B48 are also degraded because these particles associate with apo E which binds to the LDL receptor.

This mode of presecretory degradation of nascent apo B was also invoked as a possible explanation for why apo B secretion is impaired in apo E^{-/-} mice, whereas apo B secretion is increased in transgenic mice and hepatoma cells that over-express apo E. The basis for this conjecture was that apo E binds avidly to the LDL receptor and thus might compete with apo B for receptor binding, both within the secretory pathway and on the cell surface. The prediction, however, was found not to be correct because when apo E production was eliminated in LDL receptor^{-/-} mice, VLDL secretion was still 50% less than in LDL receptor^{+/+} mice (L.M. Havekes, 2001).

8. Assembly and secretion of chylomicrons

When dietary fat enters the intestine TGs are hydrolyzed to monoacylglycerols and fatty acids which diffuse across the microvillus membrane. TGs are subsequently re-synthesized in the enterocyte and large amounts of TGs are packaged with other lipids and apo B48 into chylomicrons that are secreted into mesenteric lymph. The wide variation in sizes of chylomicrons (35 to >250 nm) reflects the supply of TGs to the

intestine and affords a mechanism by which TG secretion can be readily altered in response to diet.

A cell culture model frequently used to study intestinal apo B secretion is the human colon carcinoma cell line, CaCo2, which secretes both apo B100 and apo B48 (E.J. Schaefer, 1987). In many respects, the intestinal assembly of chylomicrons is similar to the hepatic assembly of VLDLs, although there are some differences. Both apo B48 and MTP are required for normal chylomicron assembly. ER luminal TG droplets, the size of chylomicrons, but lacking apo B, were observed in enterocytes of mice lacking intestinal apo B synthesis, suggesting that chylomicron assembly might utilize these apo B-free, ER luminal TG droplets (R.L. Hamilton, 1998). Interestingly, in CaCo2 cells almost no newly synthesized apo B is degraded intracellularly. Moreover, ubiquitinylation of intracellular apo B is not detectable although CaCo2 cells do possess the machinery for adding ubiquitin to proteins for proteasomal degradation (L. Chan, 2000).

One unique feature of chylomicron assembly is revealed in individuals with chylomicron retention disease (Anderson's disease). These people secrete apo B100-containing VLDLs from the liver but do not secrete apo B48-containing chylomicrons from the intestine. This rare disease is characterized by hypocholesterolemia and lipid malabsorption [30]. Since the intestinal apo B editing mechanism is intact and apo B48 is synthesized normally, a defective gene, different from genes involved in VLDL assembly, appears to be responsible for this disease.

9. Assembly of lipoprotein(a)

In addition to its presence on VLDLs and LDLs, plasma apo B is also found in humans, some primates and hedgehogs (but not rodents) covalently bound via a single disulfide linkage to a glycoprotein called apo(a). Apo(a) is synthesized in significant quantities only in the liver and associates with apo B of LDL to form lipoprotein(a). In human plasma, the concentration of lipoprotein(a) varies from <1 to >100 mg/dl. In general, a high level of lipoprotein(a) is an independent risk factor for development of coronary artery disease (A.M. Scanu, 1992).

Apo(a) contains tandem repeats of sequences that are very similar to the 'kringle' motifs of plasminogen suggesting that apo(a) arose by duplication of the plasminogen gene. A cysteine residue (Cys-4057) in one kringle domain forms a disulfide linkage with Cys-4326 of apo B100. Apo B100 of many mammals cannot form a disulfide linkage with human apo(a). For example, in transgenic mice expressing human apo(a), this apo(a) circulates in plasma free of apo B-containing lipoproteins. A step-wise model for assembly of lipoprotein(a) has been proposed. Initially, a non-covalent interaction occurs between a lysine residue (Lys-680) in the N-terminus of apo B100 and a lysine-binding site in apo(a) (M.L. Koschinsky, 2001). Next, a non-covalent interaction between the C-terminal region of apo B100 and amino acids 4330–4397 of apo(a) occurs, followed by formation of the disulfide bond between apo B and apo(a) (S.P.A. McCormick, 2000). Most data support a model of lipoprotein(a) assembly in which apo(a) associates with apo B of LDL in the plasma after secretion, rather than within hepatocytes prior to secretion.

10. Future directions

The past few years have been marked by an explosion of data on lipoprotein metabolism obtained from genetically modified mice. These studies have been extremely useful for elucidating mechanisms of assembly and secretion of lipoproteins, particularly because there are limitations to studying this process in cultured cells. However, one must also acknowledge the limitations of using mice as models for studying human lipoprotein assembly since lipoprotein metabolism in mice is distinct in several respects from that in humans.

Several important questions remain regarding the mechanisms of lipoprotein assembly. It is surprising that although the MTP gene and protein have been so extensively studied, and although we know that an absence of MTP severely impairs the secretion of apo B-containing proteins, we still do not know exactly how this protein participates in lipoprotein assembly. In *in vitro* assays, MTP transfers lipids between membranes. Consequently, it is assumed that MTP transfers TGs to apo B. However, this lipid transfer function of MTP has not yet been demonstrated in cells. Another proposed role for MTP is in the transfer of TGs across the ER membrane to form luminal TG droplets, but how this process occurs and the composition of the droplets, are not known. There are currently no data explaining why animals make apo B100 in addition to apo B48 since apo B48-only mice appear normal. Nor do we understand why apo B is made in large excess of its requirement for lipoprotein secretion or why hepatocytes degrade a large fraction of newly synthesized apo B intracellularly in an apparently wasteful process. In addition, proteases involved in apo B degradation within the lumen of the secretory pathway remain to be identified. Several post-translational covalent modifications of apo B occur (glycosylation, phosphorylation and palmitoylation) yet we do not understand the biological relevance of these modifications. The mechanisms that regulate chylomicron assembly are poorly understood. For example, the defective gene responsible for Anderson's disease, in which intestinal apo B48/chylomicron secretion is severely impaired while secretion of apo B100 from the liver is normal, has not yet been isolated nor has its function been identified. The stage is now set for resolution of these issues within the next few years.

Abbreviations

ACAT	acyl-Co A : cholesterol acyltransferase
apo	apolipoprotein
ER	endoplasmic reticulum
LDL	low-density lipoprotein
MTP	microsomal triacylglycerol transfer protein
SREBP	sterol response element binding protein
TG	triacylglycerol
VLDL	very low-density lipoprotein

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