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SIGNPOSTS IN THE ASSEMBLY OF CHYLOMICRONS

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1. ABSTRACT

Intestinal cells synthesize and secrete chylomicrons in the postprandial state. Synthesis of these particles is defective in abetalipoproteinemia and chylomicron retention disease. Chylomicrons are very large, heterogeneous, lipid-rich particles ranging in diameters from 75 to 450 nm and function to transport dietary fat and fat-soluble vitamins to blood. The size heterogeneity of the secreted particles depends on the rate of fat absorption, type and amount of fat absorbed. The fatty acid composition of triglycerides present in chylomicrons reflects the composition of dietary fat, whereas the fatty acid composition of chylomicron phospholipids does not. The differences in the fatty acid compositions are also observed when lipids are labeled with glycerol. Thus, the differences are not due to differential incorporation of dietary fatty acids into different lipids but are mainly due to different pools of lipids used for chylomicron assembly. It has been suggested that preformed phospholipids and nascent triglycerides are preferentially used for intestinal lipoprotein assembly.

Biosynthesis of chylomicrons requires apoB48. ApoB48 is translated from apoB mRNA that is post-transcriptionally edited in the intestinal cells to incorporate a stop codon. Nascent apoB48 may be cotranslationally lipidated and this process is critically dependent on the presence of microsomal triglyceride transfer protein. Two different models have been proposed for the assembly of chylomicrons. In the independent model, intestinal cells are hypothesized to synthesize VLDL and chylomicron by two independent pathways. The chylomicron assembly pathway is hypothesized to be sensitive to a surfactant, Pluronic L81, but that of VLDL assembly is not. In the sequential assembly model, synthesis of all lipoproteins is hypothesized to begin with the assembly of apoB-containing primordial lipoprotein particles. The primordial particles are suggested to fuse with triglyceride-rich lipid droplets that are synthesized independently of apoB. This process results in the core expansion of primordial particles and the synthesis of nascent lipoproteins. Differences in the size of secreted lipoproteins may be due to differences in the size of triglyceride-rich lipid droplets. Pluronic L81 is

hypothesized to inhibit the formation of large triglyceride-rich droplets that serve as precursors for chylomicron assembly. In this review, we have discussed some signposts that might be unique to different steps in the assembly of chylomicrons. First, it is proposed that the association of preformed phospholipids with nascent apoB in the endoplasmic reticulum may serve as a signpost for the very early steps in the assembly of chylomicrons. Second, association of large amounts of newly synthesized triglycerides compared to preformed triglycerides may serve as a signpost for the assembly of larger lipoproteins. Third, the incorporation of retinyl esters may serve as markers for the final stages of chylomicron assembly. These signposts may be helpful in the identification and characterization of various intermediates in the assembly of chylomicrons. The knowledge about the molecular assembly of chylomicrons may lead to better therapeutic agents for controlling various hyperlipidemias, obesity, and atherosclerosis.

2. INTRODUCTION

Gage and Fish observed that blood obtained after a fatty meal contained large fat particles “chylomicrons” that can be observed under a high-power dark-field microscope (21). Furthermore, they showed that the presence of chylomicrons was correlated with the intake of fatty meals and not with meals rich in carbohydrates, proteins, or a combination of both (21). Thus, the appearance of chylomicrons in the blood was shown to be due to the secretion of these particles by the enterocytes in the postprandial state after the fat intake.

Apart from chylomicrons, intestinal cells secrete smaller VLDL-size particles. The assembly and secretion of VLDL, however, mainly occurs in the fasting state and during the experimental infusion of lecithin or palmitate (56,57,67). They may serve to transport lipids derived from the sloughed mucosal cells and bile, and fatty acids derived from plasma. In contrast, chylomicron assembly occurs in the postprandial state and they are the primary particles responsible for the transport of dietary fat and fat-soluble vitamins. Rapid delivery of fatty acids and efficient triglyceride synthesis may be necessary for the induction of chylomicron assembly (48).

3. PROPERTIES OF CHYLOMICRONS

3.1. Composition

Chylomicrons are large, triglyceride-rich, spherical particles (1,22). Lipids and proteins are the major and essential constituents of these lipoproteins.

3.1.1. Lipid composition

Lipids constitute ≈ 99% of the mass of these particles. The majority of lipids are triacylglycerols which constitute ≈ 85-92% of chylomicrons isolated from thoracic lymph duct of dogs fed a high fat emulsion (1,37,38,77,79). Almost all of the triacylglycerols are present in the core of the particles. The core does not appear to contain any ordered structure in electron micrographs (80). Phospholipids are the second most common lipids found in the particles and contribute ≈ 6-12% of the mass and form the outer single layer of the particles (52,79). The amount of phospholipids present in the particles is sufficient to cover ≈ 80% of the surface of chylomicrons (79). Cholesterol constitutes ≈ 1-3% of the mass and exists in free and esterified forms. Esterified cholesterol is present in the core whereas free cholesterol is present on the surface of these particles.

3.1.1. Protein composition

Even though proteins constitute ≤ 1 % of the total mass, they are essential for the assembly and secretion of chylomicrons. The proteins associated with chylomicrons are apoB48, apoAIV, apoAI, and apoCs. ApoB48 is an essential structural protein and remains associated with the particles from the biosynthesis till catabolism. In contrast, other apolipoproteins, such as apoAIV, apoAI, and apoCs, are secreted with chylomicrons and dissociate from the particles in the plasma to variable extent. The dissociation of these apolipoproteins is poorly understood. However, hydrolysis of the particles by endothelial cell-bound lipoprotein lipase may play an important role in the release of these apolipoproteins from the circulating chylomicrons. In addition, hydrolyzed chylomicrons acquire apoE from the plasma and this protein plays an important role in the removal of these particles by the liver (table 1).

3.2. Size heterogeneity and relationship with dietary fat

Chylomicrons are heterogeneous particles. Their size changes with the rate of fat absorption. The particles secreted at the beginning of fat absorption are small, at the peak of absorption are the largest, and their size decreases in the later periods of absorption (19,77). In addition, the type of fat absorbed also affects the size of the secreted particles. Infusion of either phospholipids or triglycerides results in the secretion of VLDL or chylomicrons, respectively (67). Furthermore, the amount of fat absorbed also affects the particle size. Animals on high fat diet secrete larger lipoproteins as compared to chow-fed animals (19). Thus, the heterogeneity in the size of secreted particles can be due to the rate of fat absorption, type and amount of fat absorbed (33).

3.3. Chylomicron assembly is a specific property of intestinal cells

Since apoB48 is an integral part of chylomicrons and only intestinal cells synthesize apoB48 in humans, it was believed that CM assembly was a specific property of this protein. However, over expression of recombinant human apoB48 in non-differentiated intestinal cells and non-intestinal does not result in the assembly and secretion of chylomicrons by these cells (27,31,39,49). In addition, apoB100 is secreted as part of chylomicrons by Caco-2, human colon carcinoma, cells (48). Furthermore, expression of apoB100 instead of apoB48 in the intestine of transgenic mice does not appear to affect the transport of fat and fat-soluble vitamins (18). The biochemical and biophysical characteristics of the lipoproteins secreted by the intestinal cells of these animals have not been studied. Thus, chylomicron assembly appears to be a specific property of the intestinal cells and not that of apoB48 protein (for a detailed discussion see (33)). Intestinal cells

Table 1. Differences between the metabolism of apoB gene products by intestine and liver

	Intestine	**Liver**
Location of enhancer elements for tissue-specific expression (upstream of the transcription start site (kb)	54-62	0-5
Size of mRNA (kb)	≈ 15	≈ 15
Post-transcriptional editing of the mRNA	Yes	No
Translational products	ApoB48	ApoB100
Protein size (kDa)	≈ 260	≈ 550
Amino acids	2152	4536
Lipoproteins	Chylomicrons, VLDL	VLDL, IDL, LDL, Lp(a)
Lipoprotein diameter (nm)	75-450	35-80
Floatation density (g/ml)	< 0.93	< 1.006
Svedberg's flotation units (S_f)	= 400	20 – 400
Fasting plasma levels	Very low	High
Half-life in plasma	≈ 10 min	Hours to days
Presence of Vitamin A	Yes	No
Structural protein recognized by the LDL receptors	No	Yes
Clearance requires apoE	Yes	No
Lipoprotein secretion in abetalipoproteinemia	No	No
Lipoprotein secretion in chylomicron retention disease	No	Yes

probably express unique factor(s) that are necessary for chylomicron assembly.

3.4. Transport of dietary fat

A normal adult may consume 100-150 g of triglycerides, 2 g of phospholipids, 1-2 g of cholesterol and variable amounts of fat-soluble vitamins daily. Dietary triacylglycerols, phospholipids, and cholesterol esters are first hydrolyzed in the intestinal lumen and the resulting products (monoacylglycerol, fatty acids, cholesterol) are taken up by enterocytes. Lipids are re-synthesized, packaged into lipoproteins, and secreted into the lymph by intestinal cells. Most of the lipid absorption and transport occurs within the duodenum and jejunum. However, the colon can also absorb and transport fat.

The dietary fatty acids are incorporated differentially into phospholipids and triglycerides of chylomicrons. Whyte *et al*. studied the incorporation of dietary or exogenous fatty acids into the triacylglycerols and phospholipids secreted with chylomicrons (74). They showed that the majority of the dietary fatty acids fed to rats were present in chylomicron triacylglycerols. In contrast, the addition of endogenous fatty acids into chylomicron phospholipids was in far more excess than the addition of dietary fatty acids, so that the fatty acid composition of phosphatidylcholine was relatively constant and was independent of the fat absorbed (74). They showed that intestinal cells incorporated dietary fatty acids into phosphatidylcholine, but phospholipids with dietary fatty acids were not secreted with chylomicrons. These studies indicated that there are two pools of phospholipids: phospholipids that contain dietary or exogenous fatty acids and phospholipids that contain endogenous fatty acids. Phospholipids that do not contain dietary fatty acids were present in chylomicrons. Thus, the fatty acid composition of phospholipids does not reflect dietary fatty acid composition, whereas the fatty acid composition of triglycerides present in chylomicrons reflects the fatty acid composition of dietary fat.

Similar differential incorporation of phospholipids and triglycerides into chylomicrons was observed when all the lipids were simultaneously labeled with glycerol in Caco-2 cells (48). We studied the incorporation of "preformed" and "nascent" lipid pools into secreted lipoproteins and observed that "preformed" phospholipids were preferentially used for the assembly and secretion of lipoproteins (48). In contrast to phospholipids, nascent triglycerides were preferentially used over preformed triglycerides for chylomicron assembly. Based on these studies, it has been suggested that phospholipids for intestinal lipoprotein assembly are derived from a pre-existing intracellular pool and thus do not contain dietary fatty acids. In contrast, newly synthesized triglycerides are preferentially used for chylomicron assembly and thus contain dietary lipids (for a detailed discussion, see (33)).

1. BIOSYNTHESIS OF CHYLOMICRONS

4.1. Transcription of *apob* gene

Apolipoprotein B48 is the essential protein for the assembly and secretion of chylomicrons. It is co-linear with the N-terminal 48% of apoB100 synthesized by the liver (table 1). In the human genome, there is one *apob* gene of ≈ 43 kb on chromosome 2 consisting of 29 exons and 28 introns. It is expressed mainly in the liver and intestine, and to some extent in the heart. Early experiments involving the transgenic expression of human and mouse apoB in mice revealed that the expression of apoB in the liver can be achieved by using bacteriophage clones that contained 19 kb sequence present at the 5´ end of the transcription start site (10,47,51). However, these mice did not express human apoB transgene in the intestine. Subsequently, sequences required for expression in liver and heart were shown to be present within the 5 kb upstream region of the transcription start site (54). To identify tissue-specific enhancer elements required for intestinal expression, the laboratory of Steve Young used ≈ 145 kb bacterial artificial chromosomes spanning ≈ 70 kb

of 5´ sequences, ≈ 22 kb of 3´ sequences and the entire 43 kb human apoB gene to create transgenic mice. The transgenic mice expressed human apoB in the intestine (55). Furthermore, they performed *recA*-assisted restriction endonuclease cleavage to truncate the 5´ and 3´ sequences in the bacterial artificial chromosome (54). These studies established that the tissue-specific enhancer elements for intestinal expression are present between 54-62 kb upstream of the transcription start site (54).

Transcription of the *apob* gene is believed to be constitutive. The gene is transcribed into a 15 kb mRNA in the liver and intestine (table 1). The mRNA exist in two different translatable (polysome-rich) and nontranslatable (polysome-poor) pools (14). The apoB mRNAs isolated from the cytoplasm of rat and rabbit livers exhibit unusual physical properties. When subjected to sedimentation velocity centrifugation through a linear sucrose density gradient, the majority of the apoB mRNA migrates more slowly than other actively translated mRNAs indicating unique conformational features of apoB mRNA-containing polysomes. Metrizamide equilibrium density ultracentrifugation studies demonstrated that apoB mRNA can be isolated in two different pools: one polysome-rich, translatable pool and the other polysome-poor, mRNP (mRNA protein particles). Factors that affect this distribution may have significant effects on apoB synthesis. The molecular basis for this unusual feature is not known but this behavior is not due to unusual apoB mRNA size, post-transcriptional editing, or association of the nascent polypeptide with lipids.

In the intestine, the apoB mRNA undergoes a site-specific, post-transcriptional, intra-nuclear editing (table 1). This editing was the first example of a mammalian RNA that undergoes site-selective deamination. Only the cytosine$_{6666}$ in ≈ 15 kb mRNA is deaminated to uracil by an editosome complex. This editing converts a glutamine codon at 2153 to a stop codon. There are four *cis*-elements spanning 139 nucleotides in the apoB mRNA that are important for the efficient site-specific deamination of the cytosine$_{6666}$. A “mooring sequence” 5 to 15 nucleotides downstream of the edited base is essential and sufficient for the site-specific editing. It is believed that the editing complex binds to the “mooring sequence” and seeks out the cytosine base for editing. A sequence between the edited base and the mooring sequence is the “spacer sequence”. The spacer sequence of 3-5 nucleotides is required for proper editing, i.e., cytosine immediately 5´ of the mooring is poorly edited. In addition, there are enhancer elements at the 5´ and 3´ ends of the edited base that modulate the editing activity *in vitro*. (for reviews, see (13,16,29,30,32,60,61)).

The apoB mRNA is edited by multi-component “editosomes” that have not yet been fully characterized. However, the essential enzyme responsible for the editing has been cloned (62). The cytosine deaminase, also called apobec-1, is a single polypeptide of 237 amino acids that binds to RNA. Its catalytic domain consists of His-61, Cys-93, Cys-96, and Glu-63 residues and requires Zn^{2+} for its activity. The enzyme does not require any cofactors or energy and thus is different from RNA processing enzymes that are involved in splicing and polyadenylation. The apobec-1 also contains a leucine-rich region that might be involved in homo-dimerization and protein-protein interactions with other auxiliary proteins present in the editosomes. The auxiliary proteins are necessary for the proper and specific editing of the apoB mRNA. The expression of apobec-1 is developmentally regulated in humans and rats (20,23). It is not expressed in the fetal intestine but expressed after birth. The expression of apobec-1 activity parallels the expression of apoB48 in the adult intestine. The apobec-1 expression is not altered by dietary and hormonal manipulations in the intestine (5,42,63).

4.2. Translation of apoB mRNA

In the liver, the apoB mRNA is translated into a single polypeptide of 4536 amino acids called apoB100 (table 1). In contrast, the edited mRNA in the intestine is translated into a single polypeptide of 2152 amino acids and is called apoB48 (table 1). There is evidence to suggest that the production of apoB may be controlled at the level of translation of the apoB mRNA (36). However, a major mechanism of control for the assembly of lipoproteins in HepG2 cells is believed to be intracellular degradation of mis-folded and/or inadequately lipidated apoB (76). Recently, it has been suggested that intracellular degradation may not play a significant role in the regulation of apoB secretion from Caco-2 cells (46). Thus, mechanisms controlling apoB secretion by intestinal cells need to be studied in detail.

The nascent apoB polypeptide is synthesized like a normal secretory protein. However, unlike other secretory proteins, apoB is co-translationally integrated, at least transiently, into the endoplasmic reticular membranes in a transmembrane orientation. The nascent apoB is co-translationally lipidated. Lipidation of nascent apoB may result in the release of apoB, as a “primordial lipoprotein particle”, from the endoplasmic reticular membrane into the lumen of the endoplasmic reticulum. The co-translational lipidation resulting in the formation of primordial particles appears to depend on the length of apoB polypeptide. As the length of apoB increases, there is increased lipidation of the nascent polypeptide and larger lipoproteins are formed (for detailed discussion, see (33,36)). According to this relationship, apoB48 is expected to form HDL-size particles. In fact, expression of apoB48 usually results in its secretion as HDL particles (39,49). This relationship, however, appears to be only true for the formation of primordial lipoprotein particles (33). The primordial particles undergo further lipidation resulting in the formation of nascent lipoproteins. The major differences with respect to the size of lipoproteins secreted by the intestine and liver may be due to differences in the degree of subsequent lipidation of primordial particles.

4.3. Microsomal triglyceride transfer protein and lipoprotein assembly

Extraction of nascent apoB from the membrane to the lumen of the endoplasmic reticulum in lipidated form may be critically dependent on the neutral lipid transfer

activity of microsomal triglyceride transfer protein (MTP). MTP may transfer neutral lipids to a site where apoB is associated with the ER membrane and saturate the phospholipid monolayer. Protein-protein interactions between apoB and MTP may favor the deposition of neutral lipids at a site close to apoB. This may initiate the formation of "primordial lipoprotein" that may bulge out into the lumen of endoplasmic reticulum. Further release of these particles into the lumen may be assisted by the protein-protein interactions between MTP and nascent apoB (3). However, the role of MTP in the subsequent steps of intestinal lipoprotein assembly is poorly understood.

Evidence for the presence of a neutral lipid transfer activity in the lumen of the endoplasmic reticulum was first obtained based on *in vitro* lipid transfer assays using lumenal contents of the endoplasmic reticulum (70,73). Subsequently, the protein responsible for the lipid transfer activity, the MTP, was shown to consist of 97 and 55 kDa subunits. The 55 kDa polypeptide was an ubiquitous enzyme, protein disulfide isomerase, present in the endoplasmic reticulum, whereas the 97 kDa polypeptide was unique and was required for the lipid transfer activity (for reviews, (26,72)).

The need for the 97 kDa MTP subunit in the secretion of apoB-containing lipoproteins was established by using various independent approaches. First, abetalipoproteinemia patients were shown to contain mutations in the gene encoding this polypeptide (for reviews, (26,72)). These individuals do not contain apoB in their plasma. Second, co-expression of the 97 kDa polypeptide with apoB polypeptides was shown to result in the assembly and secretion of lipoproteins in cells that do not secrete lipoproteins (25,27,43). Third, compounds that inhibit MTP's lipid transfer activity *in vitro* were identified and shown to decrease apoB secretion in cells (6,7,24,28,40,69,71).

MTP may play other roles, independent of its lipid transfer activity, in lipoprotein assembly. In Caco-2 cells, an MTP inhibitor, BMS-200150, that does not affect triglyceride synthesis has been shown to decrease triglyceride-secretion without inhibiting apoB secretion (68). The mechanism of inhibition of triglyceride secretion independent of apoB secretion by the MTP inhibitor is not known. In addition to its lipid transfer activity, MTP has been shown to bind to apoB with high affinity (2,9,34,35,50,58,75). Protein-protein interactions between these proteins decrease with increases in the length and degree of lipidation of apoB (34). Lysine and arginine residues in the N-terminal 18% of apoB were shown to be critical for MTP binding (2). Within this region, apoB and MTP can interact at multiple sites (9,50). One of the MTP binding sites has been localized to amino acids 270-570 (35). To understand the importance of apoB-MTP binding, we have identified a novel antagonist, AGI-S17, that inhibits apoB-MTP binding without affecting MTP's lipid transfer activity *in vitro* and in cells. AGI-S17 decreased apoB secretion in hepatoma cells indicating that these interactions may be necessary for the secretion of apoB-containing lipoproteins (3). The protein-protein interactions between apoB and MTP may suggest chaperone activity of MTP. Thus, in addition to its lipid transfer activity, MTP plays additional important roles in the assembly and secretion of lipoproteins.

4.4. Proposed models for the assembly of chylomicrons

4.4.1. Independent assembly of chylomicrons

Two models for the assembly and secretion of chylomicrons have been discussed in detail elsewhere (33). In the "independent" model, Tso and associates proposed that there are two independent pathways for the assembly of VLDL and chylomicrons (64,67). According to this model, VLDL assembly occurs always and chylomicron assembly occurs in the postprandial state due to induction of a new pathway that is inhibited by Pluronic L81.

4.4.2. Sequential assembly of chylomicrons (figure 1)

In the "sequential assembly" model, we proposed that the synthesis of all lipoprotein particles share a common early step that results in the synthesis of "primordial lipoprotein" particles (33,36). The assembly of primordial lipoproteins may involve the release of nascent apoB with preformed phospholipids present in the ER membrane into the lumen of the ER. It is critically dependent on the lipid transfer and chaperone activities of MTP. It has been suggested that the length of apoB polypeptide may determine the sizes of the primordial lipoproteins. The formation of primordial particles renders apoB secretion-competent.

Furthermore, it has been proposed that "triglyceride-rich lipid droplets" of various sizes are synthesized independent of the assembly of primordial lipoprotein particles. It has been suggested that the heterogeneity in the size of the "nascent lipoproteins" may be due to heterogeneity in the size of these droplets (33). The droplets may be formed due to enhanced synthesis of triglycerides in the postprandial state. Pluronic L81 may destabilize larger lipid-droplets and may inhibit the formation of these droplets and chylomicron assembly.

The triglyceride-rich lipid droplets are hypothesized to fuse with primordial lipoprotein particles to form "nascent lipoproteins" in a process called "core expansion". The core expansion refers to increase in the size of primordial particles that would occur after the fusion. This step may be crucial in rendering triglyceride-rich droplets secretion-competent and may be the rate-limiting step that determines the transport of triglycerides from the endoplasmic reticulum to the Golgi complex. It is very likely that this process occurs at a junction between smooth endoplasmic reticulum and rough endoplasmic reticulum. At present, very little is known about this process. In this model, the size of the secreted lipoproteins is proposed to be dependent on the size of the lipid-droplets formed under different conditions. In the postprandial state,

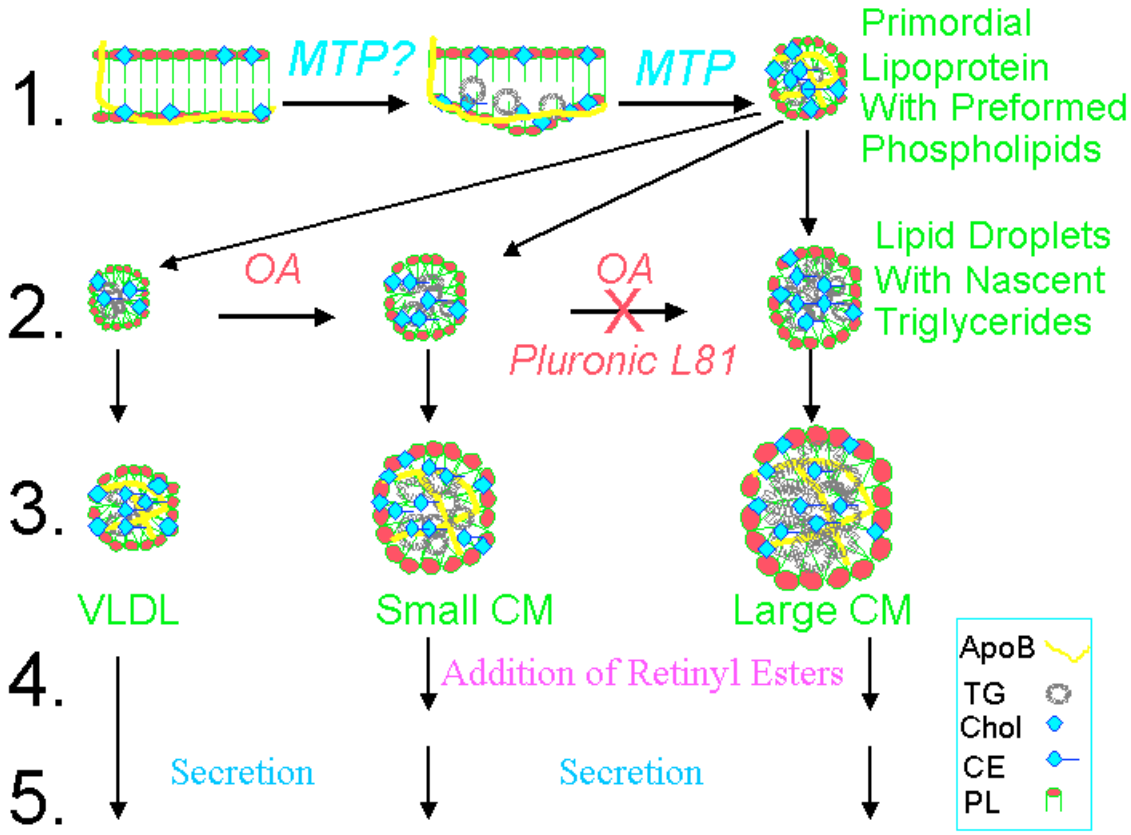

Figure 1. Biochemical signposts for different stages of chylomicron assembly. 1. Primordial lipoproteins with preformed phospholipids: The nascent apoB molecule is shown to interact with the inner leaflet of the endoplasmic reticulum membrane (59). The site of apoB/membrane interactions is depicted to start concentrating neutral lipids. This process could be mediated by MTP. After substantial transfer of neutral lipids, the apoB may bud off as a small spherical particle. In this process, phospholipids present in the inner leaflet of the endoplasmic reticulum may form the outer layer of the primordial lipoproteins. Metabolic labeling experiments show that newly synthesized phospholipids are not targeted to the site of lipoprotein assembly and "preformed phospholipids" are used for the assembly of intestinal lipoproteins (48). Thus, it is proposed that incorporation/association of preformed phospholipids with nascent apoB can be used as a signpost for this step. 2. Lipid droplets with nascent triglycerides: It is hypothesized that under basal conditions small triglyceride-rich particles are formed independent of apoB. In the postprandial state, such as increased availability of oleic acid (OA), the size of these particles increases due to the incorporation of nascent triglycerides. The formation of larger lipid droplets is hypothesized to be especially sensitive to Pluronic L81. 3. Core expansion: This step may involve fusion of primordial lipoprotein particles with triglyceride-rich lipid droplets of various sizes to give rise to lipoprotein particles of different sizes. This step is crucial for the transport of large amounts of triglycerides. 4. Addition of retinyl esters. Retinyl esters are secreted only with chylomicrons and not with other lipoproteins such as VLDL, IDL and LDL (53). Thus, they may serve as signposts for the final stages of chylomicron assembly. 5. Secretion. This step represents the secretion of nascent lipoproteins. The signals for the secretion of nascent lipoproteins are not known. Most likely, secretion of lipoproteins is a constitutive process. Incompletely assembled lipoproteins may be selectively retained and degraded. TG, triglycerides; Chol, cholesterol; CE, cholesterol esters; PL, phospholipids.

larger droplets are formed that may fuse with primordial particles to form chylomicrons. Extensive studies are required to understand various biochemical steps involved in the assembly and secretion of intestinal lipoproteins.

5. BIOCHEMICAL SIGNPOSTS FOR VARIOUS STEPS IN CHYLOMICRON ASSEMBLY

Recently, cell cultures models have been described that can help understand molecular assembly of chylomicrons. Levy and associates have used human fetal primary enterocytes, colonocytes, and crypt cells to study the assembly and secretion of intestinal lipoproteins (4,44,45). In these studies, secretion of chylomicrons by colonocytes and crypt cells was shown to be significantly low compared to the amounts of chylomicrons secreted by enterocytes. We showed that Caco-2 cells secrete chylomicrons after the supplementation of high concentrations of oleic acid in the presence of taurocholate (48). Cartwright and Higgins showed that isolated rabbit primary enterocytes supplemented with bile salt/lipid micelles secreted chylomicrons (12). Furthermore, secretion of chylomicrons by enterocytes was shown to increase by feeding high fat diets to rabbits for 2 weeks

before the isolation of enterocytes (11). These cell culture models may help us understand various molecular steps involved in the assembly of chylomicrons. Thus, at this juncture, it is prudent to assign some unique signposts to various steps involved in the assembly of chylomicrons. These signposts may be valuable in the identification and characterization of various intermediates in the assembly and secretion of chylomicrons.

5.1. Preformed phospholipids; markers for the very early steps in chylomicron assembly

To identify biochemical markers that are incorporated into all the particles, we hypothesize that the addition of lipids to nascent apoB may occur in sequential steps and these lipids may be derived from different metabolic pools. To test this hypothesis, we radiolabeled cellular lipids with radiolabeled glycerol and then followed their secretion by Caco-2 cells (48). We observed that all the different size lipoproteins secreted by Caco-2 cells preferentially used preformed phospholipids. This suggested that the synthesis of different size particles may involve a single initiation step that uses phospholipids present in the cells prior to the induction of chylomicron assembly. Most likely, this step represents the lipidation of endoplasmic reticulum associated nascent apoB with phospholipids that are present in the membranes (figure 1). This would result in the synthesis of apoB-containing primordial particles that contain preformed phospholipids. It also implies that newly synthesized phospholipids are not targeted to the ER membrane. Thus, the assembly of primordial lipoproteins may involve non-covalent lipidation of nascent apoB with the phospholipids present in the membrane of the endoplasmic reticulum and the association of preformed phospholipids with nascent apoB may serve as a signpost to identify and characterize these primordial particles.

5.2. Increased amounts of newly synthesized triglycerides; markers for the assembly of larger lipoproteins

It has been hypothesized that larger triglyceride-rich lipid droplets are synthesized in the lumen of the smooth endoplasmic reticulum during the postprandial state, and the heterogeneity in the size of secreted lipoproteins is due to differences in the size of these droplets (33). Metabolic labeling studies showed that secreted VLDL contain equal amounts of triglycerides derived from preformed and nascent pools. In contrast, chylomicrons contained substantially higher amounts of nascent triglycerides even when the pool of nascent triglycerides was smaller than that of the preformed triglycerides (48). Thus, we interpret this to suggest that higher amounts of nascent triglycerides may be used as signposts to identify chylomicrons or droplets that may serve as precursors of chylomicrons.

5.3. Retinyl esters, markers for the final stages of chylomicron assembly

We observed that retinyl esters are secreted only when Caco-2 cells assemble and secrete chylomicrons (53). In the absence of chylomicrons, retinyl esters are stored in the cells. Furthermore, these cells do not secrete retinyl esters even when significant amounts of apoB are secreted as VLDL, IDL and LDL-size particles but not as chylomicrons (53). These studies indicate that retinyl esters are mainly targeted to chylomicrons for secretion. Thus, retinyl esters may serve as signposts to identify the final stages of chylomicron assembly (figure 1).

5.4. Pluronic L81; an inhibitor for the final stages of chylomicron assembly

Pluronic L81 contains 10% hydrophilic and 90% hydrophobic residues and is a liquid at room temperature. Addition of Pluronic L81 to a high fat and high cholesterol diet for one month resulted in lower serum cholesterol and triglycerides levels, and decrease in body weight in rats (8). It was subsequently shown to decrease the secretion of triglycerides into the lymph without affecting the digestion, absorption, and re-esterification of triglycerides (66). Pluronic L81 may inhibit the synthesis of larger triglyceride-rich lipid droplets because it may reduce the surface elasticity and/or viscosity of the droplets. This would result in preferential destabilization of larger droplets and thus reduce nascent droplets that could serve as precursors for chylomicron assembly (figure 1). It should be pointed out that the larger triglyceride-rich droplets formed from nascent triglycerides are hypothesized to be especially susceptible to the action of Pluronic L81. The mature droplets such as those observed in the cytosol of animals infused with Pluronic L81 may be resistant to Pluronic L81 (65,66).

6. DISORDERS DUE TO DEFICIENCY IN CHYLOMICRON ASSEMBLY

Chylomicron assembly and secretion are severely impaired in two autosomal recessive disorders: abetalipoproteinemia and chylomicron retention disease. In abetalipoproteinemia, lipoprotein assembly is impaired in liver as well as in intestine indicating commonality in the assembly of lipoproteins by these two organs (table 1). In contrast, only intestinal lipoprotein assembly is impaired in chylomicron retention disease indicating for the existence of tissue-specific differences in the assembly of lipoproteins by these two organs.

6.1. Abetalipoproteinemia

Abetalipoproteinemia is due to mutations in the *mttp* gene (for reviews, see (26,72)). *Mttp* gene encodes for 97-kDa subunit of the MTP that has been identified and characterized as a protein that transfers lipids between vesicles. Mutations in the *mttp* gene result in loss of lipid transfer activity in the intestinal and liver cells. In these patients, lipoprotein assembly and secretion is absent both in the liver and intestine. Patients are characterized by severe malabsorption of fats and fat-soluble vitamins, stunted growth, acanthocytosis of red blood cells, peripheral neuropathy, and pigmentary retinal degeneration.

6.2. Chylomicron retention disease or Anderson's disease

The characteristic features of the chylomicron retention disease include absence of apoB48 in the plasma, steatorrhea and diarrhea, lipid-filled enterocytes, low

plasma cholesterol levels, and growth retardation. It can be distinguished from abetalipoproteinemia due to the presence of low levels of LDL, absence of red blood cell acanthocytosis, and presence of retinitis pigmentosa. It is an autosomal recessive disorder. The genetic basis of the disorder is unknown (33). Recently, Dannoura *et al*. performed a comprehensive study of 8 affected individuals in 7 unrelated families of North African origin (15). Electron microscopic studies showed lipoprotein-like particles in membrane-bound compartments and large-lipid droplets that were not surrounded by membranes. Although the endoplasmic reticulum contained VLDL and chylomicron size particles, these particles were generally absent in the Golgi complexes and intercellular spaces, suggesting that chylomicron retention disease may represent a defect in the transport of lipoproteins from the endoplasmic reticulum to the Golgi complex. Intestinal organ cultures showed that apoB and apoAIV were synthesized and secreted, albeit with poor efficiency compared with normal organ cultures, into the media as part of lipoprotein particles. Segregation analyses of 4 families excluded, as a cause of the disease, significant regions of the genome surrounding the genes of apolipoproteins AI, AIV, B, CI, CII, CIII, E, fatty acid binding proteins, and MTP. Thus, factors other than apolipoproteins and MTP are also important for the efficient assembly and secretion of intestinal lipoproteins.

6.3. Mice with chylomicron assembly deficiency

Targeted deletion of *apob* gene is lethal in mice (17). However, mice that do not synthesize apoB in the intestine but synthesize in the placenta and liver are viable (78). These mice are normal at birth. However, within 24 h they develop distended abdominal cavities due to accumulation of fat within enterocytes, and exhibit severe growth retardation. The absorption of vitamins A and E is severely impaired in these mice. The deficiency in chylomicron assembly neither affects plasma lipid levels nor hepatic lipoprotein assembly and secretion (41). In the absence of exogenous lipid supply via chylomicron pathway, the liver has been shown to use free fatty acids present in the plasma for the synthesis of triglycerides (41). Thus, intestinal lipoprotein assembly is essential for the absorption of fat and fat-soluble vitamins, and proper growth and development of neonates.

7. CONCLUDING REMARKS

Understanding of chylomicron assembly and catabolism has lagged far behind the understanding of lipoprotein assembly by the liver. As discussed in this and other reviews (33,36), important concepts are evolving about chylomicron assembly. Mechanisms involved in the assembly of larger chylomicrons from the shorter apoB48 peptide remains to be elucidated. In addition, various proteins involved in the assembly pathway needs to be identified. Cloning of enzymes involved in the intestinal biosynthesis of triglycerides will be crucial to appreciate fully the molecular assembly of chylomicrons. The knowledge about chylomicron assembly may lead to better therapeutic agents that can control hyperlipidemias, obesity, and atherosclerosis.

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Abbreviations: apo, Apolipoproteins, HDL, High density lipoproteins, MTP, Microsomal Triglyceride transfer protein, VLDL,Very low density lipoproteins; Review

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