Review Article

Shunts, channels and lipoprotein endosomal traffic: a new model of cholesterol homeostasis in the hepatocyte

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Abstract

The liver directs cholesterol metabolism in the organism. All the major fluxes of cholesterol within the body involve the liver: dietary cholesterol is directed to the liver; cholesterol from peripheral cells goes to the liver; the liver is a major site of cholesterol synthesis for the organism; cholesterol is secreted from the liver within the bile, within apoB lipoproteins and translocated to nascent HDL. The conventional model of cholesterol homeostasis posits that cholesterol from any source enters a common, rapidly exchangeable pool within the cell, which is in equilibrium with a regulatory pool. Increased influx of cholesterol leads rapidly to decreased synthesis of cholesterol. This model was developed based on *in vitro* studies in the fibroblast and validated only for LDL particles. The challenges the liver must meet *in vivo* to achieve cholesterol homeostasis are far more complex. Our model posits that the cholesterol derived from three different lipoproteins endosomes has three different fates: LDL-derived cholesterol is largely recycled within VLDL with most of the cholesterol shunted through the hepatocyte without entering the exchangeable pool of cholesterol; high density lipoprotein-derived CE is transcytosed into bile; and chylomicron remnant-derived cholesterol primarily enters the regulatory pool within the hepatocyte. These endosomal channels represent distinct physiological pathways and hepatic homeostasis represents the net result of the outcomes of these distinct channels. Our model takes into account the distinct physiological challenges the hepatocyte must meet, underlie the pathophysiology of many of the apoB dyslipoproteinemias and account for the sustained effectiveness of therapeutic agents such as statins.

Keywords: ACAT2, cholesterol, hepatocyte, HMGCR, LDLR

Cholesterol, an amphipathic four-ringed lipid molecule, was discovered in gallstones in 1784. The word-cholesterol- is derived from the ancient Greek: "chole" meaning bile and "stereos" meaning solid. Since then, cholesterol has been studied extensively, and successfully, with 13 Nobel Prizes awarded for describing its synthetic and transport pathways. Cholesterol is a critical component of biologic membranes providing stability but also fluidity in the plasma membrane of cells with no cell wall. Cholesterol is also a precursor to many biologically essential products such as steroid hormones, bile acids, vitamins, and co-factors. Some have suggested that the evolution of animals was dependent on the presence of cholesterol[1] and it is found in all eukaryotes: yeast[2], *C. elegans*[3], zebrafish[4], moths[5], and vertebrates. Because of its unique

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amphipathic nature, cholesterol must be transported through the blood packaged within lipoprotein particles. This transport is achieved by different lipoprotein particles that transport the dietary cholesterol from the intestine to the liver and from the periphery to the liver. In the early 1960s, the risk of atherosclerotic coronary artery disease was positively related to the plasma levels of total cholesterol and then low density lipoprotein-cholesterol (LDL-C). For this reason, the regulation of cholesterol transport and synthesis have been major directions of research. Clinical outcomes have improved substantially with the development and implementation of statins and our biologic understanding with the recognition and elucidation of clathrin-dependent receptor-mediated endocytosis of lipoprotein particles.

Current model of cholesterol homeostasis within the liver

The current cholesterol homeostatic models are deeply entrenched in the literature[6-17]. Brown, Goldstein, their colleagues and others constructed and explicated an explanatory model with several key steps. Embedded in the ER membrane, sterol-cleavage activated protein (SCAP) binds sterol response element binding protein (SREBP1 or SREBP2) and serves as a chaperone to both SREBPs[6]. For the present discussion of cholesterol metabolism, we will restrict our discussion to SREBP2. With lower cellular cholesterol, the SCAP/SREBP2 complex traffics through the secretory pathway where SREBP2 interacts with two separate proteases in the Golgi apparatus: Site 1 protease (S1P) and Site 2 protease (S2P)[18-19]. S1P cleaves SREBP2 removing the domain that SCAP binds to, releasing a membrane bound truncated SREBP2. Subsequent S2P interaction and cleavage releases a soluble truncation form of SREBP2 that is a transcription factor. This soluble form migrates to the nucleus and interacts with specific DNA sequences termed sterol response elements (SRE)[6]. Genes that possess SRE include, but are not limited to, the low density lipoprotein receptor (LDLR) and 3-hydroxyl, 3-methyl-glutaryl coenzyme A reductase (HMGCR). Binding of SREBP2 to the SRE of the *LDLR* gene and *HMGCR* gene promotes transcription of the mRNAs, ultimately resulting in increased LDLR protein on the cell surface and increased HMGCR protein in the ER membrane. Increased LDLR on the cell surface results in increased binding and uptake of LDL particles from the extracellular milieu, thus increasing intracellular cholesterol. HMGCR is the rate-limiting enzyme of cholesterol biosynthesis[9]. An increase in HMGCR results in an increase of *de novo* synthesized cholesterol. This two-punch combination-increased uptake and increased synthesis-increases the cellular content of cholesterol.

When cholesterol content tends to become excessive, mechanisms exist to reduce cellular cholesterol levels and restore cholesterol homeostasis. LDL is taken up by receptor mediated endocytosis in clathrin coated pits. LDLR and bound LDL are internalized within endosomes; LDLR is recycled back to the plasma membrane and LDL is degraded in lysosomes, with LDL-derived cholesterol trafficked out of the lysosome by Niemann-Pick Type C protein 1 and 2 (NPC1, NPC2). LDL-derived cholesterol is trafficked to the ER membrane, where it interacts with SCAP, strengthening SCAP's interaction with the insulin induced gene 1 (*INSIG1*) preventing the trafficking of the SCAP/SREBP2 complex to the Golgi apparatus[20-21]. Thus, no cleavage of SREBP2 occurs, no trafficking of SREBP2 to the nucleus takes place, and no transcriptional activation of SREBP2-regulated genes results. *HMGCR* and *LDLR* gene transcription are shut down, closing the cycle.

This is the core of the conventional model of cholesterol homeostasis within cells. If the mass of cholesterol within the cell increases, then endogenous synthesis of cholesterol decreases as does the synthesis of the LDLR, preventing exogenous uptake of LDL-derived cholesterol. If the mass of cholesterol within the cell decreases, endogenous synthesis of cholesterol and exogenous uptake of cholesterol is increased.

Acute regulation of both LDLR and HMGCR also occurs. Inducible degrader of LDLR (IDOL) acts as an E3 ubiquitin ligase specific for LDLR and promotes the proteolytic degradation of LDLR[17,22-23] independent of SREBP2. Ubiquitination of HMGCR also occurs although the mechanism may involve one of many identified effectors[24-26]. Likewise, gp78 is the E3 ubiquitin ligase for INSIG1[27]. Ubiquitination of squalene synthase, another key regulatory enzyme of *de novo* cholesterol biosynthesis[28-29], occurs mediated by MARCH6[30-31]. Ubiquitination of HMGCR and INSIG1 is dependent on the presence and binding of an oxysterol, such as 25-hydroxycholesterol, to each protein. This adds a level of complexity in that oxysterols may be key in regulating the over-accumulation of cholesterol in cells (for more information, please see reviews[32-33]).

Liver X receptor (LXR) forms a heterodimeric transcription factor and, in response to binding of oxysterols, binds LXR response elements (LXRE) promoting the transcription of a number of genes including ATP binding cassette transporter A1 and G1 (*ABCA*1, *ABCG1*), stearoyl Co-A desaturase, apolipoprotein E, *SREBP1c*, fatty acid synthase (*FAS*), and

IDOL[34-41]. LXR activation rids the cell of cholesterol through cholesterol efflux to high density lipoprotein (HDL) particles *via* ABCA1 and ABCG1, while actively promoting the degradation of LDLR, resulting in less cholesterol uptake. Thus, oxysterols could promote the ubiquitin-dependent degradation of HMGCR and INSIG1 (lowering endogenous synthetic levels of cholesterol), stimulate the expression of IDOL to degrade LDLR, and stimulate the expression of ABCA1/ABCG1 to promote cholesterol removal from the cell, thereby acting as an effective foil to the SREBP2 pathway. The one catch to the beneficial action of LXR is that in the liver, *FAS* and *SREBP1c* are also target genes of LXR mediated upregulation. FAS facilitates synthesis of fatty acids and SREBP1c is a master regulator of lipogenesis, which result in triacylglycerol (TAG) accumulation, generating a fatty liver, and diminishing the potential utility of LXR agonists in treatment of hypercholesterolemia.

The liver is the central organ of cholesterol uptake, regulation and export[36-41] in the organism. All the major fluxes of cholesterol involve the liver: dietary cholesterol goes to the liver; cholesterol goes from the liver to peripheral cells; cholesterol from peripheral cells goes to the liver; the liver is the major site of cholesterol synthesis for the organism. The hepatocyte is polarized having a basolateral surface in contact with the blood supply and an apical surface connected to the bile canalicula. It is very difficult to reproduce polarized hepatocytes in culture, but this has been achieved in a few studies[42-45].

Notwithstanding that the vast majority of the cholesterol flux into, through, and out of the body occurs in the hepatocyte[15] the vast majority of experiments of cellular cholesterol transport, metabolism and homeostasis have been performed in fibroblasts, which are so much easier to culture and study. The cultured skin fibroblast was the experimental model in which the principles of the regulation of intracellular cholesterol homeostasis were identified and extrapolated to all the other cells in the body. The cultured skin fibroblast was also the experimental model in which the primary molecular defect in the LDL pathway, a mutation in the LDL receptor that is responsible for familial hypercholesterolemia, was identified. However, regulation of cholesterol homeostasis in the hepatocyte appears to be substantially different than in the fibroblast, which should not be surprising given the vastly different metabolic challenges and realities these two cells face. Accordingly, we will present an updated model of hepatocyte cholesterol homeostasis, which takes these realities into account.

Cholesterol influx into the hepatocyte

Numerous lipoprotein receptors exist on the surface of the hepatocyte: LDLR, VLDLR, LRP1, LRP5/6, apoER2, scavenger receptors SR-BI, and P2Y13, as well as potentially other yet unidentified scavenger receptors. The most important is the LDLR responsible for uptake of LDL in the liver. The VLDLR, though predominantly expressed in neurons and adipose tissue, may play a role in uptake of VLDL and in TAG metabolism in the liver[46-49]. LRP1 is a type I transmembrane protein receptor with over 50 known ligands including protease inhibitor complexes (e.g. uPR), transfer proteins (e.g. lactoferrin), and signaling proteins (e.g. transforming growth factor beta)[50-54]. Importantly, LRP1 may be the primary receptor for apoE containing lipoproteins including chylomicron remnants (CR). Chylomicrons are produced in the intestine, released into the portal circulation, where they are acted upon by lipoprotein lipase primarily in adipose tissue and skeletal muscle, hydrolyzing the TAG-rich core of the chylomicrons and releasing the fatty acids to be taken up and utilized by the adjacent cells. The residual lipoprotein particle, CR, is TAG-depleted but retains its complement of cholesterol and cholesteryl ester. The liver takes up almost all of the CR suggesting that LRP1 plays a role in the uptake of the majority of CR. LRP5/6 and apoER2 (LRP8) are predominantly expressed in nervous tissue and play a major role in signaling in the Wnt pathway[55-60]. However, a role of these receptors in liver hepatocyte lipoprotein metabolism cannot be ruled out. SR-BI is a scavenger receptor that mediates selective uptake of HDL-derived cholesteryl ester (and also partially LDL-derived cholesteryl ester), but may also serve as a vehicle for efflux of free cholesterol to lipoprotein acceptors[61-62]. Knockout models and genetic variants in human populations have demonstrated that SR-BI plays a major role determining plasma HDL-levels and in the reverse cholesterol transport pathway (ie. return of cholesterol from the periphery to the liver). Therefore, SR-BI is a major contributor to the cholesterol pool of the hepatocyte. Ecto-F1-ATPase, expressed at the basolateral membrane of hepatocytes, binds HDL[63]. Subsequent activation of the purinergic receptor P2Y13 results in clathrin-mediated endocytosis of HDL. P2Y13 plays a role in HDL uptake and in sterol transport into bile[64-68]. In addition to the defined lipoprotein receptors, heparan sulfate proteoglycans are glycoproteins on the surface of cells that bind lipoproteins, and assist in their uptake by the hepatocyte[69].

The uptake of lipoproteins in the hepatocyte has

primarily been assessed by knockout mouse models. KO of the LDLR in fibroblasts virtually abolishes LDL uptake (>95% inhibition); however, KO of LDLR in the hepatocyte does not abolish LDL uptake but results in a decrease from 50 to 70%[70-74]. Heparan sulfate proteoglycans account for some but not all of the residual LDL uptake, suggesting other receptors, previously mentioned or unknown, may also be contributing[14,41,69,75-76]. Multiple KOs have also been attempted demonstrating a partial overlap of specificity and compensation of different receptors. For example, a recent study demonstrated that hepatic uptake of VLDL in the LRP1/LDLR/VLDLR triple KO is also mediated by heparan sulfate proteoglycans and SR-BI. It is for these reasons (number of different receptors and overlap of lipoprotein receptor specificity) that the hepatocyte cannot easily be compared with simpler cell models such as fibroblasts. Likewise, the mouse model system is not a perfect model of human lipoprotein metabolism (hence the attempt to derive "humanized" mouse models), and conclusions drawn from mouse KOs must be assessed in this light.

Cholesterol synthesis

The enzyme pathway that converts acetyl CoA into the 27 carbon, 4-ring structure that is cholesterol has been described in detail. The rate limiting enzymes for cholesterol biosynthesis are HMGCR and squalene synthase (also called farnesyl diphosphate farnesyl-transferase)[6,28-29]. Most of the literature has focused on HMGCR (as the majority of regulation does occur here) and so we will focus our attention here. HMGCR is regulated by insulin/glucagon and by cellular energy levels (by AMP-dependent protein kinase). At a transcriptional level, HMGCR is activated by SREBP2. At a post-translational level, HMGCR is targeted for proteolytic degradation in acute response to high cellular cholesterol levels. The liver produces about 75% of the total body cholesterol. This demonstrates that dietary cholesterol plays a significant but lesser role in total body cholesterol and that the regulation of cholesterol synthesis in the liver is the most important metabolic target. For this reason, statins which target the liver have been an effective treatment for hypercholesterolemia[6].

Hepatic cholesterol influx and efflux

The liver is the central organ for cholesterol metabolism and homeostasis. On the influx side, the liver is the major site for cholesterol synthesis in the

organism. The liver is also the major site to which cholesterol is delivered to the liver within CR, VLDL, LDL and HDL particles in amounts that are substantially larger than the capacity of the liver to secrete cholesterol within bile or as bile acids. The most obvious physiologic role of VLDL is to remove excess TAG from the liver and deliver it to adipose tissue and skeletal muscle. However, VLDL particles contain substantial amounts of co-secreted cholesterol or CE plus substantial amounts of cholesterol transferred from HDL particles by cholesteryl ester transfer protein.

We ingest between 500 mg and 1 g of cholesterol per day. We secrete between 500 mg and 1 g of cholesterol per day either as bile acids or cholesterol dissolved within the bile. At least 3-4 g of cholesterol per day return to the liver within CR, VLDL, LDL and HDL particles[77]. If the only routes of cholesterol out of the liver were dissolved in bile acids or broken down to bile acids, cholesterol would accumulate progressively, and soon unacceptably, within the hepatocyte. Secretion within VLDL particles or transfer to HDL particles are the only options to maintain the balance. Thus, microsomal triglyceride transfer protein (MTP) co-translationally loads the apoB-100 with CE as well as TAG to generate a VLDL particle[78-81]. Thus, secretion of VLDL can offload CE as well as TAG from the liver. Most of the cholesterol secreted from the hepatocyte within VLDL particles plus all of the cholesterol transferred from HDL particles to either VLDL or LDL particles returns to the liver. The VLDL secretion pathway is, therefore, largely a futile cycle without physiologic purpose so far as net movement of cholesterol in and out of the liver. The hepatocyte produces the highest level of any cell type of the ATP binding cassette transporter ABCA1 which, with the help of apoA-I, generates nascent HDL particles, which can remove cholesterol from the hepatocyte.

Regulatory intracellular cholesterol pool

The cytosolic enzyme, acylCoA:cholesterol acyl-transferase (ACAT or stearoylCoA-O-acyltransferase (SOAT)), converts the amphipathic free cholesterol, the biologically active form of cholesterol, which regulates the activity of the SREBP2 pathway, to the hydrophobic CE, the biologically inactive form of cholesterol, which must then be stored within a lipid droplet to sequester its hydrophobicity[7,12]. The storage capacity of lipid droplets is substantial in many cell types (for example macrophages that become foam cells). CE hydrolases (CEH) are also present to provide ready access to stored CE, if necessary[7].

The regulatory pool represents a pool of cholesterol

that serves as a reservoir for cellular needs, but, more importantly, as the driver of the mechanisms to regulate the total cellular cholesterol content of the cell[6,9,15]. If there is too little cholesterol, mechanisms are activated to rectify the shortfall. If there is too much, then alternate mechanisms are activated to rectify the excess. Regulation of ACAT/CEH activity can control the regulatory pathways of cholesterol within the cell. The actual physical site of the regulatory pool is likely the ER membrane. The SCAP/SREBP2 complex is in the ER membrane. HMGCR is found in the ER membrane. VLDL is synthesized in the ER lumen. It is safe to say that the ER membrane is where the action is occurring.

However, the ER membrane is characterized by very low levels of cholesterol. This is advantageous for regulation of cholesterol by SCAP/SREBP, HMGCR and ACAT, as cholesterol levels can be maintained at a low threshold that when exceeded can be promptly detected and reacted to[82-84]. However, the plasma membranes, not the ER membranes, are the location of the bulk of free cholesterol in the cell. Furthermore, all the major physical pools of cholesterol (plasma membrane, mitochondria, lipid droplet, TGN, ER membrane) are either in close contact or have access to transport mechanisms that allow rapid equilibrium or functional accumulation. Moreover, cholesterol can be removed from the regulatory pool and stored as CE or released from this compartment to re-enter the regulatory pool. This is the conventional model of cholesterol homeostasis.

Hepatocyte cholesterol homeostasis

Unlike the simple model of cholesterol homeostasis described in fibroblasts[6], the liver model of homeostasis is much more complicated. There are multiple mechanisms of influx of cholesterol and efflux of cholesterol as well as complex regulation of endogenous synthesis. Furthermore, the LDLR is regulated by a protein called proprotein convertase subtilisin/kexin type 9 (PCSK9)[13,40,47-48,85]. PCSK9, which is synthesized and secreted by hepatocytes, binds the LDLR on its ligand binding domain, leading to the internalization of the LDLR:PCSK9 complex and their subsequent degradation in the lysosome, rather than allowing LDLR's recycling back to the plasma membrane. Paradoxically, PCSK9 is regulated by SREBP2, which can lead to a confounding effect of low cellular cholesterol levels leading to upregulation of HMGCR, LDLR (increasing cellular cholesterol levels through endogenous and exogenous pathways) and upregulation of PCSK9 (decreasing cell surface levels of LDLR). Hepatocytes also express two types of ACAT (in

contrast to fibroblasts): ACAT1 (SOAT1) and ACAT2 (SOAT2)[12]. These proteins are independently regulated and serve two separate physiologic functions. ACAT1, expressed at nominal levels and unregulated, is responsible for the cytosolic pool of CE generation stored in the lipid droplets, which is intimately connected to the regulatory pool. ACAT2, which is strongly inducible, produces CE dedicated to VLDL secretion[86]. The source of cholesterol for ACAT2 is not known, since it is unlikely that ACAT1 and ACAT2 share a substrate pool. In addition to these hepatocyte specific features of cholesterol homeostasis, a number of observations in hepatocytes have argued against the cholesterol homeostatic mechanism described above.

1) LDLR is not downregulated upon LDL uptake in hepatocytes: Even in patients with hypercholesterolemia (high plasma LDL-C), the LDLR is still expressed on the surface of hepatocytes[70-74]. Under high LDL-C conditions, LDL uptake is continuous and each hepatocyte should have amassed a large surplus of intracellular cholesterol[87]. According to the conventional model of cholesterol homeostasis, LDL-derived cholesterol that is taken up by hepatocytes should enter the regulatory pool, should inactivate SREBP2, and over time, should shut down synthesis of the LDLR as it does in fibroblasts[6]. However, this does not occur and this suggests that the hepatic LDLR is regulated in a different fashion by cholesterol and other factors[86,88-99]. On the other hand, uptake of CR and the accompanying cholesterol results in a significant downregulation of the LDLR.

2) HMGCR activity and endogenous cholesterol synthesis is not downregulated by uptake of LDL. LDL-derived cholesterol should bind SCAP preventing SREBP2 trafficking and processing, leading to a downregulation of HMGCR expression, especially under chronic high LDL-C conditions. However, endogenous synthesis of cholesterol and HMGCR activity remain elevated in high LDL condition[71]. On the other hand, and again in contrast to LDL-uptake of CR-derived cholesterol results in a significant repression of endogenous cholesterol synthesis.

3) If LDL-derived cholesterol is not affecting LDLR or HMGCR expression, then LDL-derived cholesterol is not entering the regulatory pool[72,100]. On the other hand, CR-derived cholesterol significantly downregulates LDLR and HMGCR expression and clearly enters the regulatory pathway. Accordingly, we postulate that in hepatocytes LDL-derived cholesterol and CR-derived cholesterol go to different intracellular locations.

4) LDL uptake occurs in the absence of LDLR: Familial hypercholesterolemia (FH) is characterized by

defective functioning LDLR. Nevertheless, LDL is still taken up by hepatocytes[95,101-102]. LDL can be taken up by receptors other than the LDLR (LRP1, LRP5/6, Sort1)[14,48,51,57,61,76,103-109]. In addition, several researchers have postulated the occurrence of a low affinity, unsaturable binding site mediating uptake of LDL[102,110]. It was postulated that this binding site may account for up to 30% of total uptake (in the presence of LDLR) and a higher proportion under high plasma cholesterol conditions. However, it is not known what the fate of the LDL-derived cholesterol would be when LDL is taken up by these different pathways.

5) PCSK9 is the main regulator of hepatocyte LDLR expression levels, not SREBP2. In the fibroblast, the SCAP/SREBP2 mechanism is the primary regulator of LDLR levels notwithstanding IDOL's function. In addition, there are acute mechanisms to regulate cholesterol levels (discussed above). However, in the hepatocyte, PCSK9 expression is the primary regulator of LDLR cell surface expression[13,40,47,85]. Therefore, the coincident upregulation of PCSK9 may have the most profound effect on LDLR but not HMGCR.

6) VLDL secretion is a major outlet for cholesterol from hepatocytes: Lipid availability is a prerequisite for VLDL secretion. Both TAG and CE are essential components and inhibition of either is sufficient to inhibit VLDL secretion[78,80,111-116]. Furthermore, experiments with an ACAT2-specific inhibitor resulted in a diminishment of VLDL secretion[117-120], but, interestingly, increase in ABCA1 expression and efflux of cholesterol to HDL[121] and fecal excretion of cholesterol[122]. Accordingly, we posit that VLDL secretion is a major outlet for cholesterol from the hepatocyte and can be enhanced under conditions of excess hepatocyte cholesterol.

7) LDL-derived cholesterol is a substrate for ACAT2: There is evidence that CR-derived cholesterol is preferentially esterified by ACAT1 through its interaction with the regulatory pool whereas LDL-derived cholesterol is preferentially esterified by ACAT2[86]. Importantly, when an ACAT inhibitor is added to LDL-treated cells, the LDL-derived cholesterol does invoke a regulatory effect on LDLR and HMGCR (similar to CR-derived cholesterol) suggesting that esterification of LDL-derived cholesterol is an essential step in its redirection from the regulatory pool. These observations imply different physical intracellular locations of the active sites of ACAT1 (ER-cytosolic facing) and ACAT2 (ER-lumen facing). They also suggest separate intracellular trafficking itineraries of LDL-derived cholesterol and CR-derived cholesterol.

8) LDL-derived cholesterol is preferentially resecreted within VLDL: Since ACAT2 provides the substrate CE for VLDL secretion and LDL-derived cholesterol is a preferential substrate for ACAT2, then it is postulated that LDL-derived cholesterol is preferentially shunted into an ACAT2 accessible pool for secretion within VLDL. Experimental observations have demonstrated this in a primary hamster hepatocyte model but need to be confirmed in other models[86].

Taken together, these observations provide evidence for a "shunt" pathway in which LDL-derived cholesterol does not enter the regulatory cholesterol pool but instead bypasses it by being esterified by ACAT2-dependent fashion. The newly formed CE then becomes associated with newly synthesized apoB100 and is secreted with VLDL particles. By this metabolic route, LDL-derived cholesterol cycles through hepatocytes without ever entering the regulatory pool (*Fig. 1*). Because it does not enter the regulatory pool, synthesis of LDLR and HMGCR is not downregulated.

Metabolic rationale for the shunt pathway

Why would a shunt pathway exist? One possibility is that the biologic challenge of a high plasma LDL is a relatively modern development. Previously in our evolutionary history, we must have eaten animal products rarely to the point that our bodies conserved cholesterol, a point reinforced by the very efficient recycling of bile acids and cholesterol in bile. Recently, we farmed animals and began consuming larger amounts of cholesterol containing animal products: milk, eggs, and meat. Accordingly, cholesterol and fatty acid intake increased. Increased delivery of dietary fatty acids to the liver leads to not only to increased TAG synthesis but also to increased synthesis of cholesterol. As the amount of cholesterol accumulated in our bodies, so did the cholesterol found in LDL. Instead of LDL delivering cholesterol to peripheral tissues (forward cholesterol transport), the vast majority of LDL was taken back up by the liver[74]. Our biology simply did not evolve to deal with accumulating plasma LDL, with the liver left to deal with most of the cholesterol burden. Why would a hepatocyte recycle LDL-derived cholesterol without allowing that cholesterol to interact with the regulatory pool? The answer may lie in the unique position that the liver plays in total body cholesterol homeostasis. Peripheral cells have a limited capacity to take up LDL particles. The hepatocyte does not. Even without any LDL receptors, as in patients with homozygous familial hypercholesterolemia, LDL particles will be removed by the liver by non-specific internalization. In compensation for this unfavorable position, the hepatocyte has tools to deal with cholesterol. Since it cannot limit its intake of choles-

terol, the hepatocyte transforms biologically active cholesterol to biologically inert CE by ACAT. Also, hepatocytes transform free cholesterol to CE and then export it within VLDL. Moreover, the hepatocyte expresses the highest level of apoA-I and ABCA1 promoting cholesterol release to HDL. The hepatocyte secretes cholesterol and bile acids (derived from cholesterol) directly into the bile. In this way, one could envision a substantial capacity to survive excess cellular cholesterol levels.

The biologic irony is that the organ is protected at the cost of the organism. The increased secretion of VLDL particles by the liver leads to increased numbers of LDL particles accumulating in the plasma compartment and the increased number of LDL particles drives the atherosclerotic process within the arterial wall. The net result is that the liver is protected but at a potentially fatal cost to the organism.

HDL-bile acid channel

Many researchers demonstrated that SR-BI mediates selective uptake of CE from HDL (reviewed in[62,123]). SR-BI on the basolateral membrane of hepatocytes
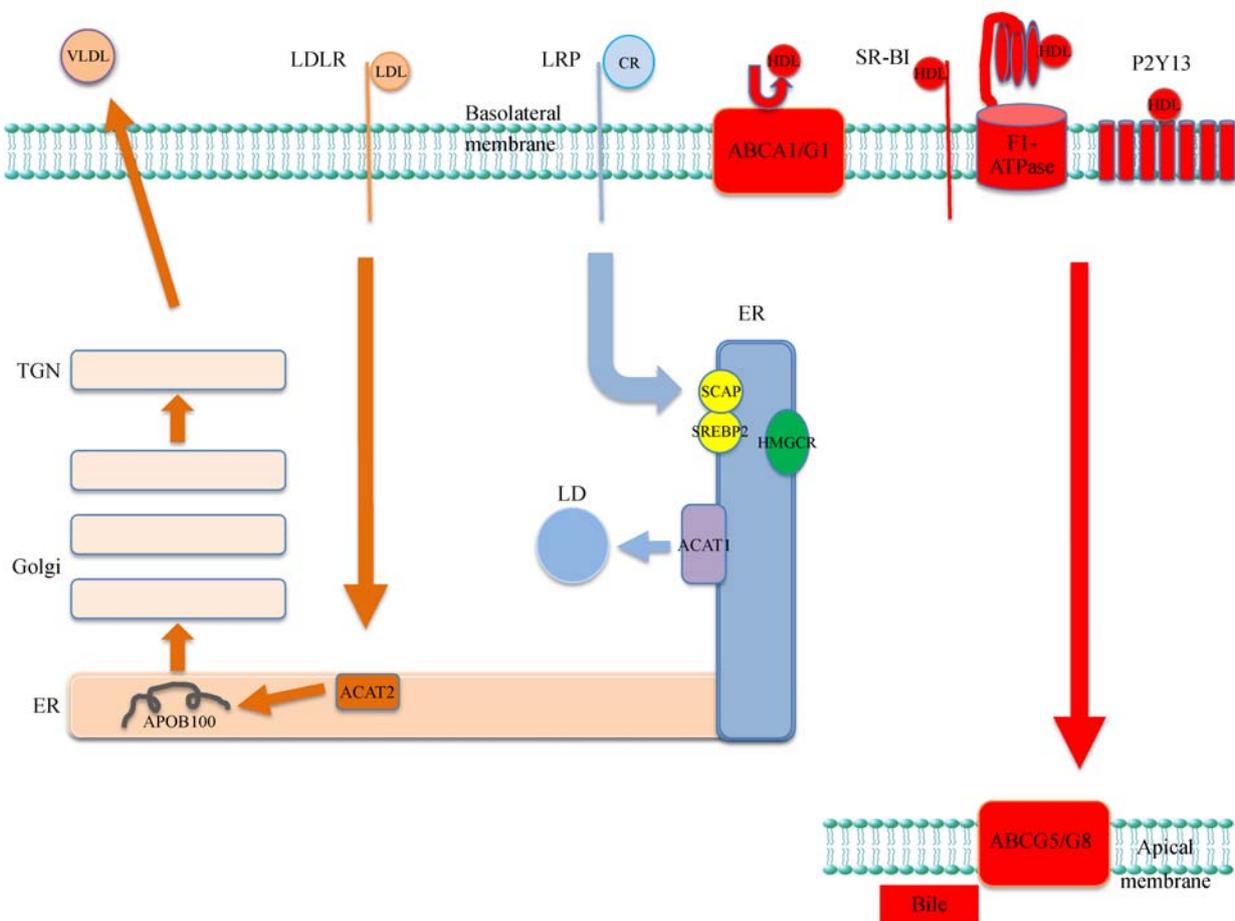

Fig. 1 Endosomal transport channels and regulation of lipoprotein-derived cholesterol in hepatocytes. In many models, it was thought that cholesterol from all sources would enter a common regulatory pool before subsequent trafficking and regulatory steps. We present evidence here that supports a model where there is independent uptake, trafficking and regulation of cholesterol taken up from LDL, chylomicron remnants (CR) and HDL. HDL-derived cholesterol is taken up by SR-BI or ecto-F1-ATPase/P2Y13 (right side), directed to the apical surface of the plasma membrane and released into the bile. CR-derived cholesterol (from the diet; middle section) is directed to the ER membrane to interact with SCAP (so-called "regulatory" pool) to prevent release of SREBP2, thereby preventing upregulation of transcription of LDLR and HMGCR (among others). Excess cholesterol in the ER membrane can be esterified to CE by ACAT1 and stored within a cytosolic lipid droplet (LD). LDL-derived cholesterol is directed to a subdomain of the ER where the cholesterol is esterified by ACAT2. This CE is directed toward the lumen of the ER to interact with apoB-100 forming a precursor VLDL particle. Upon sufficient lipidation, the VLDL is secreted. Since the LDL-derived cholesterol bypasses SCAP (or other elements of the regulatory pool), we have termed this a shunt pathway. ABCA1/ABCG1 mediate cholesterol efflux to form HDL and reduce the cholesterol load in hepatocytes. Interestingly, cholesterol efflux and HDL biogenesis and then reuptake of HDL by SR-BI or P2Y13 may not represent a futile cycle if that cholesterol is redirected to bile acid secretion. All of these pathways represent channels with independent effectors mediating trafficking and regulation, with independent effects on intracellular cholesterol homeostasis.

internalizes only CE (not the whole particle) and that CE must be hydrolyzed to free cholesterol. Then, the cholesterol is preferentially trafficked to the apical surface for secretion into bile[124-126] (***Fig. 1***). We have only a hint as to the mechanism of transport of cholesterol via transcytosis[127]; however, it is accepted in the literature as a channel specific for HDL-derived CE. In addition, HDL-apoA-I binds to the ecto-F1-ATPase expressed at the basolateral membrane of hepatocytes and stimulates the hydrolysis of extracellular ATP to ADP[63,128]. The extracellular ADP generated then selectively activates the P2Y13 purinergic receptor, resulting in cytoskeleton reorganization and subsequent clathrin-dependent endocytosis of whole HDL particles. *P2Y13*-knockout mice displayed impaired biliary cholesterol secretions[64,67-68] and were prone to atherosclerosis on apoE-KO background[65], consistent with the role of P2Y13 in HDL endocytosis by hepatocytes. Conversely, overexpression of P2Y13 in mice is atheroprotective[66]. These observations support the work of Robins and Fasulo[129] describing that HDL, but not other lipoproteins, provide a vehicle for sterol transport to bile. Together, they represent a channel as an independent trafficking itinerary for HDL-derived cholesterol.

Endosomal transport channels for lipoprotein cholesterol

The evidence we have reviewed points to specific endosomal transport channels within hepatocytes for the different lipoprotein particles that are taken up by hepatocytes (***Fig. 1***). CR-derived cholesterol enters the regulatory pool of cholesterol (causing the down-regulation of synthesis of cholesterol and the LDLR). This is also true of cholesterol delivered via VLDL, β-VLDL, chylomicron, or through non-lipoprotein means. Therefore, all these lipoproteins, besides HDL and LDL, deliver their cholesterol to the plasma membrane/ regulatory pool. LDL-derived cholesterol preferentially enters the VLDL secretory pathway, not the regulatory pool (therefore the cholesterol within this endosome has little effect on cholesterol and LDLR synthesis). HDL-derived cholesterol is preferentially trafficked to the apical surface for secretion into bile (***Fig. 1***). Importantly, when one of these channels is blocked or inhibited, another channel is turned on[86,121-122]. This suggests that there can be overlap or compensation under conditions where one channel is blocked. Therefore, we postulate the existence of lipoprotein-specific channels that direct cholesterol to a specific location with differential outcomes (***Fig. 1***). However, simply by the fact that we can identify the lipoprotein-derived

cholesterol specific channels suggests that these channels constrain the direction and flow and incoming cholesterol.

Discussion

The hepatocyte is the epicenter of whole body cholesterol homeostasis and, accordingly, faces unique and evolving metabolic changes. The endosomal transport model posits that the cholesterol derived from three different lipoproteins endosomes has three different fates: LDL-derived cholesterol is largely recycled into VLDL, HDL-derived CE is transcytosed into bile, and CR-derived cholesterol enters the regulatory pool. These channels represent distinct physiologic fates for cholesterol and create a new model of cholesterol homeostasis within the hepatocyte. These channels may have great physiologic relevance, as in human subjects with high plasma LDL-C, where one would expect that these pathways play a major role in determining plasma LDL-C levels and hepatocyte cholesterol levels.

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