Intracellular Cholesterol Transport

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Abstract—Intracellular cholesterol transport is essential for the maintenance of cholesterol homeostasis. Many aspects of cholesterol metabolism are well-known, including its synthesis in the endoplasmic reticulum, its extracellular transport in plasma lipoproteins, its uptake by the low-density lipoprotein receptor, and its regulation of SREBP and LXR transcription factors. These fundamental pathways in cholesterol metabolism all rely on its proper intracellular distribution among subcellular organelles and the plasma membrane. Transport involving the ER and endosomes is essential for cholesterol synthesis, uptake, and esterification, whereas cholesterol catabolism by enzymes in mitochondria and ER generates steroids, bile acids, and oxysterols. Cholesterol is a highly hydrophobic lipid that requires specialized transport in the aqueous cytosol, involving either vesicles or nonvesicular mechanisms. The latter includes hydrophobic cavity transporters such as StAR-related lipid transfer (START) proteins. Molecular understanding of intracellular cholesterol trafficking has lagged somewhat behind other aspects of cholesterol metabolism, but recent advances have defined some transport pathways and candidate proteins. In this review, we discuss cholesterol transport among specific intracellular compartments, emphasizing the relevance of these pathways to cholesterol homeostasis. **(*Arterioscler Thromb Vasc Biol*. 2004;24:1150-1160.)**

Key Words: intracellular cholesterol transport ■ cholesterol metabolism ■ START proteins

Cholesterol is an essential component of mammalian cell membranes, but its excess is toxic and contributes to several diseases, notably atherosclerotic vascular disease. Understanding of cholesterol homeostasis and its fine regulation has developed in several stages.[1] Early biochemical studies by Bloch et al elucidated the multi-enzyme pathway of cholesterol synthesis. Other studies described extracellular cholesterol transport by plasma lipoproteins, such as low- and high-density lipoproteins (LDL and HDL), and their effects on atherosclerosis. Brown and Goldstein et al showed that cellular cholesterol exerts negative feedback on cholesterogenic enzymes and LDL receptors via sterol regulatory element-binding protein (SREBP) transcription factors. In recent years, focus has shifted to cellular cholesterol efflux and reverse transport, whereby cholesterol moves from peripheral cells to the liver for elimination. Oxysterol derivatives of cholesterol are ligands for liver X receptor (LXR) transcription factors, which stimulate ATP-binding cassette transporters (ABCA1, ABCG1, ABCG5/ABCG8) and other genes involved in reverse cholesterol transport.[2]

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Many known pathways in cholesterol metabolism require transport of this highly hydrophobic lipid among intracellular compartments. Most cellular cholesterol resides in the plasma membrane (PM), where it constitutes 35% to 45% of lipid molecules.[3] To reach the PM and other compartments, cholesterol must exit the endoplasmic reticulum (ER), where it is synthesized, cytosolic lipid droplets, where cholesterol esters are stored, and endocytic compartments, where uptake occurs. Cholesterol transport is also essential for its effects on transcription: it must reach the ER to regulate SREBPs, and oxysterols must be generated in mitochondria and elsewhere to regulate LXRs. Understanding of intracellular transport has lagged behind other aspects of cholesterol metabolism, and this field represents a research frontier.

There are 2 general ways that cholesterol can move intracellularly, vesicular and nonvesicular.[3] Cholesterol is present in the membranes of intracellular vesicles that shuttle among compartments. Vesicular traffic typically requires an intact cytoskeleton, the tracks along which vesicles move, and ATP, providing energy for motor proteins. Although some cholesterol transport pathways are vesicular, others persist when vesicles are blocked. Nonvesicular transport can be mediated by diffusible carrier proteins, which have hydrophobic cavities to bind cholesterol and transport it across the aqueous cytosol. An example is the steroidogenic acute regulatory protein (StAR), the prototype for the StAR-related lipid transfer (START) gene family. StAR is a cholesterol transport protein that stimulates the mitochondrial conversion of cholesterol to steroids.[4] Another form of nonvesicular transport may involve spontaneous desorption of cholesterol from one membrane and diffusion to another closely juxtaposed membrane, perhaps brought together at contact sites by specialized proteins.

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There are marked asymmetries in cholesterol concentration among intracellular membranes, despite vesicular and nonvesicular transport that might be expected to equilibrate cholesterol distribution. Vesicles may have sorting mechanisms to exclude or incorporate cholesterol,[5] and intracellular transfer proteins may have specificity in targeting. Specificity could arise from interactions with receptor proteins on target membranes. Alternatively, membranes may have intrinsic differences in ability to accept cholesterol,[3] because cholesterol has highest affinity for membranes enriched in sphingolipids and saturated phospholipids.[6] It is uncertain how intracellular gradients between compartments are formed and maintained, and how cholesterol moves with and against these gradients.

ER Cholesterol Transport

The ER is the crucial regulatory compartment in cholesterol homeostasis, despite being a cholesterol-poor organelle. The ER is the primary site of cholesterol synthesis and esterification, and recent data indicate that excess free cholesterol may exert its cytotoxic effects via the ER.[7] The surface areas of the PM and ER are similar in many cells, yet much more cholesterol is in the PM. Methods to determine the cholesterol content of various cellular membranes are subject to technical limitations, but it is commonly cited that 65% to 80% of total cellular cholesterol is in PM, whereas only 0.1% to 2% is in ER.[3,5,8] ER cholesterol levels can fluctuate widely: perturbations resulting in modest ≈50% changes in PM cholesterol result in large 10-fold changes in ER cholesterol.[9] Transport between ER and PM is dynamic, because it has been estimated that the entire PM cholesterol-pool cycles to the ER and back with a half-time of 40 minutes.[10]

SREBPs Regulate Cholesterol Synthesis

SREBP transcription factors are a homeostatic mechanism whereby cellular cholesterol levels exert negative feedback on cholesterol synthesis.[11] There are 3 SREBP proteins: SREBP-2 primarily activates genes involved in cholesterol synthesis, whereas SREBP-1a and SREBP-1c have greater effects on genes involved in fatty acid synthesis. SREBPs are synthesized as transcriptionally inactive ER transmembrane proteins. When cholesterol is abundant, SREBPs remain in the ER associated with the escort protein SCAP (SREBP cleavage activating protein) and the ER retention protein Insig (Figure 1A).[12] Low cholesterol causes a conformational change in the sterol-sensing domain of SCAP,[13] dissociating Insig and allowing SREBP-SCAP to reach the Golgi. Two proteases in the Golgi release the active form of SREBP, which translocates to the nucleus to activate transcription of target genes. Cholesterol synthesis is also regulated posttranscriptionally: high cholesterol accelerates degradation of HMG CoA reductase (HMGR), the rate-limiting enzyme in cholesterol synthesis, by promoting association of its sterol-sensing domain with Insig.[14] The final enzyme of cholesterol synthesis, 7-dehydrocholesterol reductase (DHCR7), also has a sterol-sensing domain[15] and may be similarly regulated.

Cholesterol Precursor Transport

In the cholesterol synthetic pathway, cyclization of squalene generates lanosterol, the first sterol intermediate. Lanosterol is modified in 19 steps by 9 enzymes to generate cholesterol, resulting in a number of other precursor sterols, some of which have physiological functions.[16] Like cholesterol, these precursors may also require intracellular transport. The ER is the primary site of sterol synthesis,[17] but other compartments like peroxisomes and the PM may be involved. Some cholesterogenic enzymes localize to peroxisomes,[18] and there is conflicting data on cholesterol synthesis in various models of peroxisome deficiency. In peroxisome-deficient fibroblasts from humans with Zellweger syndrome, different reports have indicated decreased or unchanged cholesterol synthesis rates.[19] In mouse models lacking peroxisomal assembly (PEX) genes, cultured PEX5-deficient cells show wild-type cholesterol synthesis rates,[20] whereas PEX2-defient mice showed tissue-specific increases and decreases in cholesterol synthesis.[19] Therefore, loss of peroxisomes fails to globally inhibit cholesterol synthesis, but in these models peroxisomal enzymes mislocalize to the cytosol, where their activity and regulation may be altered.[19] The PEX2-deficient mice also showed reduced plasma and liver cholesterol levels, as well as dysregulation of various genes involved in cholesterol metabolism, suggesting that peroxisomes play some undefined role in cholesterol homeostasis. The PM may also play a role in cholesterol synthesis, because in some cell types precursor sterols reach the PM with cholesterol but at different rates.[21] Precursors like zymosterol in the PM rapidly

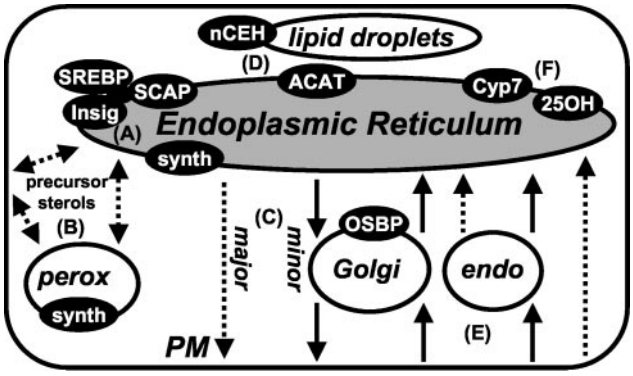

Figure 1. Endoplasmic reticulum (ER) cholesterol transport. Pathways that are likely vesicular are indicated by solid arrows, whereas candidate nonvesicular pathways are indicated by dashes. A, SREBPs and their associated regulators, SCAP and Insig, are ER proteins that constitute a homeostatic mechanism to regulate cholesterol synthesis and uptake (see text). Cholesterol synthetic enzymes (synth) also localize to the ER. B, A subset of cholesterol synthetic enzymes also localize to peroxisomes (perox), and cholesterol precursor sterols reach the plasma membrane (PM) and return to the ER. This indicates that precursor sterols shuttle among ER, PM, and peroxisomes. C, Newly synthesized cholesterol moves from ER to PM via a major Golgi-independent nonvesicular pathway, whereas a minor vesicular pathway through the Golgi also contributes. D, Cholesterol esterification by ACAT occurs in the ER. The resulting cholesterol esters are stored in cytosolic lipid droplets and are hydrolyzed by neutral cholesterol ester hydrolase (nCEH). E, PM cholesterol returns to ER for esterification via multiple pathways, with some vesicular involving the Golgi apparatus or endosomes (endo), and some nonvesicular. F, Other cholesterol-metabolizing enzymes also localize to ER, including cholesterol 7α-hydroxylase (Cyp7) and cholesterol 25-hydroxylase (25OH). The oxysterol-binding protein (OSBP) of unknown function localizes to the Golgi.