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Invited Review

Intracellular cholesterol trafficking

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Summary. The overall picture of intracellular cholesterol trafficking is very complex. The transfer of cholesterol within the cell depends on the contribution of several trafficking mechanisms. The known elements of cholesterol trafficking machinery include clathrin-coated pits, scavenger receptor type B1, caveolae, phospholipd rafts, Niemann-Pick C disease protein, sterol carrier protein 2, multidrug resistance protein, microsomal triglyceride transfer protein and steroidogenic acute regulation protein. Several pathways of intracellular cholesterol trafficking, for example retroendocytosis and cholesterol absorption in the intestine, are yet to be connected to specific structural elements. The contribution of different pathways depends on cell type, the source and destination of cholesterol and cellular cholesterol content and requirements. Some pathways are found in most, if not all, cell types, while others are associated with the specialized function of a particular cell type, for example, lipoprotein assembly in the liver or intestine and steroid hormone synthesis in steroidogenic tissue. Certain routes of intracellular cholesterol trafficking are heavily backed up by several auxiliary pathways, others entirely depend on a single functional element. In this review we describe the intracellular machinery involved in the intracellular transfer of cholesterol and give an overview of both the general and specialized pathways of intracellular cholesterol trafficking known to date.

Key words: Cholesterol, Intracellular transport, Atherosclerosis, Lipids

Abbreviations: ACAT, acetyl:cholesterol acyltransferase; CE, cholesteryl esters; ER, endoplasmic reticulum; HDL, high density lipoprotein; HMG-CoA, 3-hydroxymethyl-glutaryl coenzyme A; LDL, low density lipoprotein; MDR, multidrug resistance protein; MTP, microsomal triglyceride transfer protein; NPC, Niemann-Pick C disease; PM, plasma membrane; SCP-2, sterol carrier protein 2; SR-B1, scavenger receptor type B1; SREBP, sterol regulatory element binding protein; StAR, steroidogenic acute regulatory protein; TGN, *trans*-Golgi network; VLDL, very low density lipoprotein.

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I. Introduction

Cholesterol is an essential part of the membranes of most animal cells. Apart from maintaining the correct structural and physico-chemical properties of the membrane (Gibbons et al., 1982; Reinhart, 1990), the concentration of cholesterol in the membrane is related to a number of regulatory mechanisms (Wang et al., 1994; Shimano et al., 1996). Although rarely seen, deficiency of cholesterol is associated with the inhibition of cell growth, while the more common excessive accumulation of cholesterol is associated with a number of pathological conditions, most notably atherosclerosis, an underlying cause of most cardiovascular diseases.

There are two sources of cell cholesterol: endogenous cholesterol biosynthesis and uptake of cholesterol from plasma lipoproteins. Both these sources deliver cholesterol in either the free form of cholesterol or as cholesteryl esters (CE). Cholesterol biosynthesis from acetate or other cholesterol precursors produces free cholesterol or if esterified by acetyl:cholesterol acyltransferase (ACAT), cholesteryl esters. Exogenous lipoproteins carry and deliver to cells both free cholesterol and cholesteryl esters. Free cholesterol can be converted to cholesteryl esters both intracellularly and extracellularly, while hydrolysis of cholesteryl esters to free cholesterol only occurs intracellularly. All pathways related to cholesterol supply are very tightly regulated in response to cellular cholesterol content (Dietschy et al., 1993).

In addition to controlling delivery of cholesterol to cells, cells also regulate the amount of cholesterol removed (Fielding and Fielding, 1995a, 1997). Hepatocytes are the only cells capable of catabolizing cholesterol, converting it to bile acids, a process controlled by the enzyme cholesterol 7α-hydroxylase (Russell and Setchell, 1992). Hepatocytes, enterocytes and, to a lesser extent, macrophages can release cholesterol with newly synthesized lipoproteins. Adrenals and gonads can convert cholesterol into steroid

hormones, but this process responds to hormonal stimuli and is unrelated to cholesterol homeostasis; hormone production has little effect on cell cholesterol content. For most extrahepatic cells, however, the only way to remove excess cholesterol is to release it to a plasma acceptor, principally high density lipoprotein (HDL), a process termed "cholesterol efflux".

The ability of cells to control both the influx and efflux of cholesterol depends on the accumulation of free cholesterol in specific cellular compartments. For example, accumulation of free cholesterol in the nuclear envelope and endoplasmic reticulum (ER) results in the release of membrane bound sterol regulatory element binding protein (SREBP) as a soluble form which initiates the down-regulation of transcription of a number of genes involved in cholesterol influx, including the low density lipoprotein (LDL) receptor (regulates delivery of LDL cholesterol), and 3- hydroxy-methyl-glutaryl coenzyme A (HMG-CoA) reductase (the rate-limiting enzyme of cholesterol biosynthesis) (Wang et al., 1994). SREBP also up-regulates transcription of genes involved in cholesterol efflux, e.g. caveolin (Bist et al., 1997). Accumulation of free cholesterol in the ER stimulates cholesterol esterification, as ACAT activity is most likely dependent on substrate availability (Chang et al., 1997), while accumulation of cholesterol in specific regions of the plasma membrane results in an increased availability of cholesterol for extracellular acceptors and enhanced cholesterol efflux (Fielding and Fielding, 1997). The stability of intracellular cholesterol distribution is dependent on mechanisms such as described above, and requires a system for the effective and controlled transfer of cholesterol from one cellular compartment to another in response to changing metabolic situation. Current knowledge about the mechanisms involved in trafficking of free cholesterol inside the cells is the subject of this review.

II. Structural elements of cholesterol trafficking machinery

The uneven distribution of cholesterol among intracellular compartments and its limited solubility in water, incompatible with the actually observed rates of cholesterol trafficking, makes it unlikely that cholesterol is mobile within the cell unaided. Several proteins, protein complexes and subcellular structures have been suggested to be involved in intracellular cholesterol trafficking.

Clathrin-coated pits (vesicular transport 1).

Structure

Clathrin-coated pits are invaginations of plasma membrane which contain the protein clathrin. Clathrin comprises a structure called triskelion which consists of three 192-kDa heavy chains, each heavy chain being bound to two 30-kDa light chains. Triskelions can self-assemble to form polyhedral structures called cages. During assembly other structural components are added to the pits, namely adaptor or assembly proteins, AP1 and AP2. Adaptor proteins consist of two heavy subunits (100 kDa), two medium subunits (50 kDa) and two light subunits (19 kDa). Triskelions and adaptor proteins form a polyhedral basket, uniformly coating the intracellular surface of the membrane invagination. Another essential component of clathrin-coated pits is a 100-kDa GTPase called dynamin which is required for vesicle budding (Schmid, 1997). Clathrin-coated pits have a relatively low content of cholesterol and sphingolipid (Fielding and Fielding, 1997).

Direction

Clathrin-coated pits are involved in trafficking of cholesterol from the plasma membrane to the lysosomes (Brown and Goldstein, 1986) and *trans*-Golgi network (TGN) (Fielding and Fielding, 1996).

Mechanism

Clathrin-coated pits are central to three different pathways of cholesterol trafficking. The first pathway involving LDL-receptor mediated endocytosis is well established and has been described in several excellent reviews (Goldstein et al., 1985; Brown and Goldstein, 1986; Innerarity, 1991). In brief, after LDL binds to the LDL receptor, the ligand-receptor complex is sequestered into clathrin-coated pits. The events that follow are common to other receptors associated with clathrin-coated pits: redistribution of dynamin to the neck of the pit, budding off of coated vesicle, release of clathrin, adaptor proteins and receptor with the formation of an uncoated vesicle which may then be converted into a lysosome (Goldstein et al., 1985; Schmid, 1997). Since most LDL cholesterol is esterified, this pathway delivers mainly cholesteryl esters to the lysosomes where they are hydrolyzed and the free cholesterol is released into the lysosomal compartment.

While receptor-mediated endocytosis of lipoproteins is mainly associated with LDL receptor dependent transport, high density lipoprotein (HDL) has also been found associated with clathrin-coated pits and endosomes in HepG2 and Caco 2 cells (Garcia et al., 1996; Klinger et al., 1997). Several observations however make it unlikely that the HDL-containing endosomes are a part of a genuine receptor-mediated endocytic pathway: i) Niemann-Pick disease type C (NPC) mutation which blocks the transport of cholesterol from the lysosomal compartment completely inhibits the appearance of LDL derived, but not HDL derived cholesterol in the plasma membrane (Shamburek et al., 1997) and ii) no HDL receptor has been found associated with the clathrin-coated pits. HDL-containing endosomes may rather be an element of retroendocytosis which may be a part of the cholesterol efflux pathway (Kambouris et al., 1990) (see below).

A third pathway initiated by the interaction with clathrin-coated pits is selective transport of free cholesterol from LDL particles without internalization of the particle itself (Fielding and Fielding, 1995b, 1996, 1997). This pathway does not require binding of LDL to the receptor but is sensitive to N-ethylmaleimide (Fielding and Fielding, 1995b), monensin and cytochalasin (Fielding and Fielding, 1996). LDL derived free cholesterol is incorporated into clathrin-coated dense vesicles (Fielding and Fielding, 1996) implying that the normal endocytic pathway is required. The end-point of this pathway is however different from LDL receptor dependent endocytosis: LDL derived free cholesterol is not found in lysosomes, but rather appears in the *trans*-Golgi network (Fielding and Fielding, 1996).

2. Scavenger receptor type B1 (HDL receptor)

Structure

Hamster scavenger receptor type B1 (SR-B1) is a single polypeptide of 509 amino acids (MW 57 kDa). It has two transmembrane domains and shows a high degree of homology with CD36 (Acton et al., 1994). Murine SR-B1 and its human analog, CLA-1, have similar structures (Liu et al., 1997; Murao et al., 1997; Webb et al., 1997). Abundance of SR-B1 is highest in adrenals, ovaries, testis, adipose tissue (murine, but not human), liver, lung and monocytes (Actonet al., 1994, 1996; Landschulz et al., 1996; Murao et al., 1997).

Direction

From lipoproteins docking to the plasma membrane to the intracellular compartments (Acton et al., 1996), most likely to the Golgi (Reaven et al., 1996), and possibly from the plasma membrane to the lipoproteins (cholesterol efflux) (Ji et al., 1997).

Mechanism

A function established for SR-B1 is mediation of a process called "selective uptake of cholesteryl esters". Selective uptake of CE was first described by Pittman et al. (1987) and comprises an uptake of CE from lipoproteins, mainly high density lipoproteins, without the parallel uptake of lipoprotein particles. Several lines of evidence support the suggestion that SR-B1 mediates this process.

1. SR-B1 is most abundant in steroidogenic tissues, utilizing HDL-derived cholesterol for synthesis of steroid hormones. It is also present in liver, where HDL-derived cholesterol is used for bile acid synthesis (Acton et al., 1994, 1996; Landschulz et al., 1996; Murao et al., 1997).
2. Selective uptake of cholesteryl esters and HDL binding were significantly enhanced in cells transfected with SR-B1 (Acton et al., 1996).
3. Steroid hormone synthesis is inhibited by an antibody against SR-B1 (Temel et al., 1997).
4. In SR-B1 knockout mice, plasma cholesterol concentration was significantly increased while adrenal gland cholesterol concentration was decreased (Rigoti et al., 1997).
5. Adenovirus-mediated hepatic overexpression of SR-B1 in mice results in the virtual disappearance of plasma HDL and a substantial increase in biliary cholesterol (Kozarsky et al., 1997).
6. Estrogen treatment dramatically reduced SR-B1 content in the liver but increased SR-B1 content in the adrenal gland and corpus luteal cells of the ovary. These estrogen-induced increases in SR-B1 content in the adrenal gland and ovary were accompanied by enhanced *in vivo* uptake of fluorescent lipid from HDL (Landschulz et al., 1996). SR-B1 expression in adrenal glands *in vivo* was also induced by adrenocorticotropic hormone and suppressed by glucocorticoids (Rigotti et al., 1996).

While most of the evidence points to the role of SR-B1 in the delivery of cholesterol to the cells, it may also be involved in cholesterol efflux. SR-B1 was recently found to be localized in caveolae (Babitt et al., 1997) (see below) and overexpression of SR-B1 stimulated cholesterol efflux from cultured cells (Ji et al., 1997).

The mechanism involved in selective uptake of CE is unknown. Most likely it is a nonspecific process involving the extraction of CE from HDL attached to the cell surface, into the plasma membrane compartment (Rinninger et al., 1993) as neither plasma membrane proteins or apolipoproteins are essential for the process (Morrison et al., 1994). Further movement of CE inside the cells also occurs by unknown mechanisms, and apparently does not require protein factors nor intact cell cytoskeleton and has limited dependence on energy (Reaven et al., 1996). The role of SR-B1 in this context is most likely limited to the binding of HDL to the plasma membrane and enhancing efficiency of CE transfer ("docking" receptor) (Steinberg, 1996). Similarly, the role of SR-B1 in cholesterol efflux may also be explained by enhanced binding of HDL to the caveolae region of the plasma membrane.

It must be noted that despite being defined as a HDL receptor, SR-B1 does not have a high specificity toward HDL or apolipoprotein A-I. SR-B1 can bind native LDL, acetylated and oxidized LDL, maleylated bovine serum albumin (Acton et al., 1994). The binding of lipoproteins to SR-B1 can be equally well mediated by apoA-I, apoA-II and apoC-III (Xu et al., 1997). Also, most of the work with SR-B1 has been performed in mice, where HDL is the predominant plasma lipoprotein and the main cholesterol carrier. The function of SR-B1 may be different in humans where LDL carries most of the cholesterol in blood.

3. Caveolae

Structure

Caveolae are non-coated vesicles 50-100 nm in diameter, localized close to or attached to the plasma

membrane and containing caveolins, mainly caveolin-1 (Couet et al., 1997; Okamoto et al., 1998). Caveolin-1 is a 20-24 kDa plasma membrane protein, which has a hairpin structure with both carboxyl- and amino terminus facing the cytoplasm. Caveolin can undergo self-association forming clusters 25-30 nm in diameter (Couet et al., 1997; Okamoto et al., 1998). Caveolin is essential and probably sufficient for the assembly of caveolae: transfection of insect SF21 cells with caveolin results in formation of caveolae (Li et al., 1996). Other proteins have been found to be associated with caveolae, although it is not known if the association is permanent or transient. These proteins include signaling proteins (e.g. PKCα, casein kinase, Src-like kinases Rab5, Ha-Ras kinase, PI 3-kinase, 14-3-3, MAP kinase, adenylate cyclase), cytoskeleton proteins (e.g. myosin and spectrin), G-protein coupled receptors (e.g. ßAR and CCK-R), growth-factor receptors (e.g. EGF-R and PDGF-R) and some other receptor proteins (e.g. IP3R and CD 36) (Couet et al., 1997; Okamoto et al., 1998). Association of caveolae with SR-B1 has been recently demonstrated (Babitt et al., 1997). Most of these proteins directly interact with caveolin-1, specifically with the region between amino acids 82-101, called scaffolding domain, while others (like PDGF-R or EGF-R) require cofactors. Another essential characteristic of caveolae is a very high content of free cholesterol and sphingolipids (Simonescu et al., 1983).

Caveolae are not present in all cells. Cells like endothelial, pulmonary and smooth muscle cells, adipocytes and fibroblasts, with low levels of high-affinity LDL receptor, have a high abundance of caveolae, while cells like hepatocytes and enterocytes, with high levels of LDL receptor, have a low abundance of caveolae (Fielding and Fielding, 1997). Transformed cells usually do not express caveolin and have few or no caveolae; transfection of these cells with caveolin results in formation of caveolae on their plasma membrane and a partial retraction of their transformed phenotype (Engelman et al., 1997). However, cells without caveolin and caveolae still possess regions of the plasma membrane which share some properties of the caveolae, such as high concentration of cholesterol, sphingolipids and lipid-modified signaling molecules. These regions were called "caveolae-related domains", but their true relation to caveolae is unclear (Okamoto et al., 1998).

Direction

From the *trans*-Golgi network and endoplasmic reticulum to the plasma membrane (Fielding and Fielding, 1996; Smart et al., 1996).

Mechanism

The suggestion that caveolae are involved in intracellular cholesterol trafficking is based on the following evidence.

1. Caveolin can bind free cholesterol (Fielding et al., 1997). Moreover, complexing of caveolin with cholesterol is essential for the formation of caveolin oligomers, caveolae and rafts (see below) (Scheiffele et al., 1998).

2. Caveolae and caveolin are involved in a number of pathways related to intracellular trafficking, for example they participate in the transport of molecules across the endothelial cells and in potocytosis (Couet et al., 1997). Homooligomers of caveolin-1 and hetero-oligomers with another member of caveolin family, caveolin-2 are essential for sorting newly synthesized proteins to the basolateral or apical plasma membrane in MDCK cells (Scheiffele et al., 1998).

3. Caveolae mediate cholesterol efflux to HDL (Fielding and Fielding, 1995c).

4. Cholesterol transport to the cell surface in cells expressing caveolin was much faster than in cells lacking caveolin (Smart et al., 1996; Uittenbogaard et al., 1998).

5. Progesterone blocked movement of both caveolin and cholesterol from the ER to the PM (Smart et al., 1996).

6. Caveolin gene is regulated in response to cell cholesterol content (Fielding et al., 1997); this regulation is apparently mediated by sterol regulatory element-like sequences (Bist et al., 1997).

7. Oxysterols coordinately regulate both caveolin expression and cholesterol transfer from inside the cell to the plasma membrane (Fielding et al., 1997).

8. Association with caveolae of a large number of proteins involved in cell signaling and organized in "preassembled signaling complexes" (Okamoto et al., 1998) may suggests that caveolae are involved in signal transduction.

Fielding and Fielding (1997) suggested two models for the involvement of caveolae in intracellular cholesterol trafficking. First, caveolin may be a free cholesterol "receptor" which accepts intracellular cholesterol destined for efflux and releases it to an extracellular acceptor. In this model caveolin itself does not carry cholesterol across the cell, rather its function is limited to the sorting of cholesterol being delivered to the plasma membrane. Second, caveolin may be a part of a cholesterol ferrying complex directing delivery of cholesterol to specific domains of the plasma membrane. One of these complexes may be rafts (see below). Uittenbogaard et al. (1998), have recently described a caveolin-chaperone complex which consists of caveolin, heat-shock protein 56, cyclophilin 40, cyclophilin A and cholesterol. Disruption of this complex by cyclosporin or rapamycin prevented rapid transport of cholesterol from the TGN to the plasma membrane, and transfection of caveolin into cells which normally do not express it restored both formation of the complex and rapid transport of cholesterol (Uittenbogaard et al., 1998). It is unclear whether caveolin-chaperone complex also binds phospholipid and forms a raft, or it is an alternative caveolin-dependent ferrying complex.

4. Rafts

Structure

Rafts are defined as detergent-insoluble parts of the plasma membrane or intracellular bodies with a specific lipid content and organization. They consist of tightly packed sphingolipids. These sphingolipids are laterally associated, probably through interaction between the carbohydrate heads of the glycosphingolipids. The space between sphingolipids is filled with cholesterol. Clusters of sphingolipid and cholesterol behave as an assembly within the exoplasmic leaflet of the plasma membrane where intervening fluid regions are occupied by unsaturated phosphatidylcholine molecules, or form intracellular bodies, most likely *en route* to or from plasma membranes (Simons and Ikonen, 1997). Rafts can bind a variety of proteins including GPI-anchored proteins, transmembrane proteins, tyrosine kinases and most interestingly, caveolin (Simons and Ikonen, 1997; Scheiffele et al., 1998). Polarized epithelial cells are characterized by a high abundance of rafts (Simons and Ikonen, 1997), but their existence in other cell types can not be excluded (Yoshimori et al., 1996).

Direction

Between *trans*-Golgi network and plasma membrane (Fielding and Fielding, 1997; Simons and Ikonen, 1997).

Mechanism

It is recognized that rafts are involved in the biosynthetic transport of a number of membrane proteins from the TGN to the PM, especially to the apical membrane of polarized cells (for review see (Simons and Ikonen, 1997)). Rafts represent a convenient transport vehicle: instead of directing each protein separately to a specific region of the plasma membrane, proteins to be delivered to the same part of the membrane are incorporated into a raft which is then targeted to the apical or basolateral membrane. Since rafts are rich in cholesterol and contain caveolin, Fielding and Fielding (1997) suggested that rafts may play a role in transferring cholesterol from the TGN to the PM. Thus, caveolin may direct cholesterol-rich rafts to specific regions of the plasma membrane and then recycle back to the TGN, thus providing a continuous flow of cholesterol-rich membranes from the TGN to the plasma membrane. Alternatively, caveolin may promote specific docking of rafts to preexisting caveolae (Fielding and Fielding, 1997). Rafts are also involved in endocytic traffic carrying proteins from clathrin-coated regions of the plasma membrane independently of the vesicular transport (Simons and Ikonen, 1997) and potentially they can transport cholesterol in that direction too. It must be noted, however, that participation of rafts in cholesterol trafficking has not been demonstrated directly and it is premature to assess its contribution to the overall trafficking of cholesterol.

5. Niemann-Pick C Disease protein

Structure.

Neimann-Pick C disease is an autosomal recessive, neurovisceral lipid storage disease (Loftus et al., 1997). A human gene responsible for Niemann-Pick C disease and its murine analog have been only recently identified (Carstea et al., 1997; Loftus et al., 1997). Little is known about the product of this gene: it is a 1278-amino acid protein with homology to the morphogen receptor PATCHED, SREBP cleavage activating protein and HMG-CoA reductase (Carstea et al., 1997; Loftus et al., 1997).

Direction

From the lysosomes to the plasma membrane and endoplasmic reticulum (Loftus et al., 1997; Underwood et al., 1998).

Mechanism

At the cellular level Neimann-Pick C disease is characterized by an inability to transfer cholesterol and sphingomyelin from lysosomes to the cytoplasm. Skin fibroblasts from Neimann-Pick C disease patients as well as Chinese hamster ovary cell mutant with a phenotype similar to the NPC mutation are characterized by: i) accumulation of cholesterol and sphingomyelin in lysosomes, ii) inability to esterify LDL-derived cholesterol, iii) inability to use LDL-derived cholesterol to support cell growth, iv) lack of cellular response to an increase of cellular cholesterol content, by incubation with LDL (Liscum et al., 1989; Dahl et al., 1994). To measure cholesterol trafficking *in vivo* Shamburek et al. (1997) measured the appearance of labeled free cholesterol in the plasma and bile after injecting labeled cholesteryl linoleate. The appearance of LDL-derived, but not HDL-derived or newly synthesized cholesterol in cell plasma membrane was retarded in patients with NPC. An inability of NPC cells to transport cholesterol out of lysosomes could be mimicked by treatment of cells with monensin and nigerecin (Sakuragawa et al., 1994) and U18666A (Liscum and Faust, 1989), although it is unclear how specific the effects of these compounds are. In addition to lysosomal deficiency, peroxisomal impairment was observed in the murine model (Schedin et al., 1997).

The final destination of cholesterol exiting from lysosomes was initially identified as plasma membrane, with all other sites, including the ER, being supplied with LDL-derived cholesterol after it had passed through the plasma membrane compartment (Lange et al., 1993, 1997). However, several recent observations make it more likely that LDL-derived cholesterol can be independently delivered to both the PM and the ER.

1. Two types of somatic CHO cell mutants were selected, both exhibiting phenotypes identical to NPC fibroblasts. One of these mutants was characterized by impaired transport of cholesterol from the lysosomes to the PM, while another by the impairment of the transport from the lysosomes to the ER (Dahl et al., 1994).

2. Transport of cholesterol from lysosomes to the PM and the ER can be differentially inhibited (Underwood et al., 1998).

3. LDL cholesterol esterified by ACAT is not diluted by plasma membrane cholesterol (Underwood et al., 1998).

4. In NPC patients lysosomal cholesterol is utilized for the bile acid synthesis despite block of the cholesterol transfer to the PM (Shamburek et al., 1997).

It remains to be established if the product of the NPC gene is a specific carrier (or a part of a carrying complex) of cholesterol from lysosomes to both destinations, or whether some of the effects of the NPC mutation are indirect. Interestingly, heterozygous, but not homozygous NPC fibroblasts, have a 10-fold elevation in the level of caveolin (Garver et al., 1997) which may reflect either a compensatory mechanism or a link between caveolin-dependent and NPC gene dependent cholesterol transport.

6. Sterol carrier protein 2.

Structure

Sterol carrier protein 2 (SCP-2) is a single peptide of 123-amino acids (MW 13.2 kDa) (Vahouny et al., 1987; Yamamoto et al., 1991; Ohba et al., 1994). It is synthesized from the sterol carrier protein-x (SCPx) gene using an alternative initiation site. SCP-2 is synthesized as a 15.3 kDa precursor with subsequent cleavage of the 20 amino acid propeptide (Yamamoto et al., 1991). SCP-2 is found in all tissues with the highest concentrations in enterocytes, hepatocytes and adrenocortical cells (Vahouny et al., 1987). Intracellularly, most SCP-2 is found in peroxisomes (Keller et al., 1989). SCP-2 binds cholesterol with a Kd of approximately 0.3 μM (Colles et al., 1995), an activity attributed to the region between amino acids 100-104 (Seedorf et al., 1994).

Direction

It is unlikely that SCP-2 has a specific effect on cholesterol transport between any particular locations. Rather it non-specifically stimulates cholesterol transport by other cholesterol trafficking pathways (see below).

Mechanism

Because of the high concentration of SCP-2 in steroidogenic tissues it was originally proposed that SCP-2 carries cholesterol to the sites of steroid hormone synthesis, i.e. to the mitochondria and across the mitochondrial membrane (Vahouny et al., 1987). More recent observations have provided new information on the role of SCP-2 in intracellular cholesterol trafficking.

1. Overexpression of SCP-2 in rat hepatoma cells was associated with increased rates of transfer of newly synthesized cholesterol to the PM with a corresponding decline in cholesterol esterification (Baum et al., 1997). In contrast, transfection of SCP-2 in fibroblasts increased utilization of plasma membrane cholesterol for esterification i.e. in this case SCP-2 promoted cholesterol trafficking in the opposite direction (Murphy and Schroeder, 1997).

2. Transport of newly synthesized cholesterol from the ER to the PM is significantly reduced in SCP-2 deficient fibroblasts (Zellweger syndrome) and also after transfecting normal fibroblasts with SCP-2 antisense oligonucleotide (Puglielli et al., 1995). However, in two other SCP-2 deficient cell lines, CHO-derived ZR-82 and Reuber H35 hepatoma cells, no deficiency in cholesterol esterification or transport of cholesterol to and from the PM was observed (van Heusden et al., 1985; Liscum and Dahl, 1992).

3. SCP-2 antisense treatment reduced and delayed the appearance of newly synthesized cholesterol in the bile (Puglielli et al., 1996).

4. In *in vitro* experiments, addition of SCP-2 stimulated transfer of cholesterol between isolated mitochondrial, microsomal and plasma membranes without affecting direction of transfer (Frolov et al., 1996a,b).

5. Expression of SCP-2 is regulated in response to cholesterol loading of cells (Hirai et al., 1994; Kraemer et al., 1995).

The diversity in the effects of overexpression and deficiency of SCP-2 in different cells makes it unlikely that SCP-2 is a *bona fide* cholesterol carrier. Rather it can facilitate processes common to different pathways of cholesterol trafficking, for example, changing the structure of the membrane domains and facilitating desorption of cholesterol. It was recently demonstrated that *in vitro*, SCP-2 facilitates cholesterol transfer between membrane fractions independently whether or not they are separated by a dialysis membrane which is impermeable for SCP-2 (Woodford et al., 1995). Affinity of SCP-2 binding to cholesterol is consistent with its role as an enhancer of cholesterol desorption acting on the surface of the membrane (Colles et al., 1995).

7. Multidrug resistance protein

Structure

Multidrug resistance protein 1 (MDR1) is a member of a family of several multidrug resistance proteins (or P-glycoproteins), but only MDR1 is involved in cholesterol trafficking (van Helvoort et al., 1996). A glycoprotein of 1280 amino acids, containing 12 transmembrane domains, MDR1 is located on the

plasma membrane and parts of the protein are apparently exposed extracellularly since they react with antibody, and are available for photoaffinity labeling (Gottesman and Pastan, 1993). MDR1 has ATPase activity and two ATP binding sites, one centrally and the other at the carboxyl-terminal end of the molecule, which is a characteristic of the family of Traffic ATPases. MDR1 is expressed in most, if not all, tissues with the highest level of expression in adrenal cortical cells, renal proximal tubule epithelium, hepatocytes, enterocytes, pancreatic ductules, capillary endothelium, placenta, pregnant endometrium, lymphocytes and bone marrow (Gottesman and Pastan, 1993). Characteristically, all these cells have very active transport pathways. There is also a very high expression of MDR1 in a number of tumors which make them resistant to chemotherapy, a fact to which MDR owns its name.

Direction

Across the plasma membrane (Gottesman and Pastan, 1993) and possibly from the plasma membrane to the endoplasmic reticulum (Metherall et al., 1996; Debry et al., 1997).

Mechanism

MDR1 exhibits a broad specificity and can bind and transport a variety of substances, which have functionally little in common but all possess an amphipathic structure (van Helvoort et al., 1996). This has led to the suggestion that its main physiological function may be detoxification, but there is a strong possibility that MDR1 is involved in numerous transport pathways, including cholesterol trafficking.

The mechanism of MDR action has been described as an "ATP-dependent flippase" (Gottesman and Pastan, 1993; van Helvoort et al., 1996): MDR1 binds target molecules on the cytoplasmic leaflet of the plasma membrane and flips it across the membrane to the exoplasmic leaflet of the plasma membrane where molecules diffuse to the surrounding aqueous phase. The transport is energy dependent and can proceed against a concentration gradient.

MDR1 affects cholesterol homeostasis in a number of ways. First it may facilitate transfer of cholesterol to the outer surface of the plasma membrane. However, there is no evidence so far that inhibition of MDR affects cholesterol efflux and cholesterol can apparently move relatively freely across the plasma membrane unaided. Metherall et al. (Metherall et al., 1996; Debry et al., 1997) demonstrated that inhibition of MDR1 by progesterone led to the inhibition of cholesterol biosynthesis and esterification, a finding which they interpret as inhibition of the trafficking of cholesterol and/or its precursor from the PM to the ER where cholesterol biosynthesis and esterification occur. Progesterone however, affects a number of cholesterol trafficking pathways and it seems likely that MDR inhibition of cholesterol synthesis is indirect. Overall, despite being a non-specific lipid translocase which can transport cholesterol, there is insufficient evidence that MDR1 plays a significant role in cholesterol homeostasis.

8. Retroendocytosis (vesicular transport 2)

Structure

Retroendocytosis is another segment of the vesicular transport system. It represents the recycling branch of the transport which started in clathrin-coated pits and is a pathway whereby uncoated endosomes, with or without receptors, return to the plasma membrane. It is also possible that retroendocytosis may in part represent vesicles which are formed in the TGN to transport proteins from the Golgi to the PM, and may also carry cholesterol. However, the properties of transfer of cholesterol from the TGN to the PM are not compatible with general vesicular transport, at least in a normal physiological state (Fielding and Fielding, 1997).

Direction

From the intracellular membranes and lipid-storage droplets to the plasma membrane and extracellular compartments (Schmitz et al., 1985b; Hornick et al., 1997).

Mechanism

Retroendocytosis was originally described by Schmitz et al. (1985b) for mouse peretonial macrophages. Schmitz et al. demonstrated that HDL can specifically bind to the surface of macrophages and undergo endocytosis. However, some of the endosomes avoid the usual pathway which includes fusion with lysosomes and subsequent hydrolytic degradation, but rather are re-secreted back to the extracellular space. It was suggested that these endosomes interact with the margins of intracellular lipid droplets and remove cholesteryl esters from droplets, incorporating them into HDL (Schmitz et al., 1985b). Another report, however, suggested that retroendocytosis is involved in the delivery of cholesterol to cells (DeLamatre et al., 1990). In addition to macrophages, a similar form of retroendocytosis pathway was demonstrated for hepatocytes (DeLamatre et al., 1990; Kambouris et al., 1990), enterocytes (Herold et al., 1994) and adrenocortical cells (Fidge et al., 1984). There are several observations that support the existence of retroendocytosis.

1. Retroendocytosis is dependent on the presence of apolipoprotein A-I and A-II which makes it more likely to be a specific process (Herold et al., 1994).

2. Retroendocytosis is regulated in response to loading cells with cholesterol (Schmitz et al., 1985b; Kambouris et al., 1990).

3. Retroendocytosis is inactive in macrophages from

patients with Tangier disease, a condition known to affect cholesterol efflux. Interestingly, in Tangier disease internalization of HDL by macrophages is not impaired, but instead of being recycled, HDL-containing endosomes fuse with the lysosomal compartment (Schmitz et al., 1985a).

4. Retroendocytosis is both temperature- and energy-sensitive (DeLamatre et al., 1990), and inhibited by monensin, an inhibitor of endocytic pathways, but not by chroroquine, a lysosomal inhibitor (Kambouris et al., 1990).

It must be noted, however, that retroendocytosis was not observed in other reports (Jackle et al., 1993) and that the general methodology for studying retroendocytosis, i.e. labeling of HDL with gold or ^{125}I, is artifact prone, due to the dissociation of the label from HDL particles. It is not known whether retroendocytosis is a pathway specific for macrophages, hepatocytes and enterocytes (coincidentally, the only three cell types capable of lipoprotein synthesis and secretion) or if it is a more general phenomenon. The molecular mechanisms of retroendocytosis are also poorly understood.

Another possibility for endosomes to participate in intracellular cholesterol trafficking is their ability to to carry cholesterol in their membranes. Hornick et al. (1997) have recently demonstrated that the free cholesterol content of retrosomes (recycling component of endocytic pathway) is twice that of endosomal compartments from which they are derived. Retrosomes therefore may potentially be vehicles for transferring cholesterol from intracellular compartments to the PM.

A phenomenon which may be related to retroendocytosis has been recently described by Hoeg et al. (1990). When synthesis and secretion of apoB-100 by human intestine was studied *in situ*, the amount of apoB-100 secreted with lipoproteins exceeded the amount of apoB-100 synthesized by the intestine which opens a possibility that some lipoproteins are assembled from exogenous plasma apoB-100 following internalization by enterocytes.

9. Diffusion of cholesterol

As free cholesterol is partially soluble in water, passive diffusion through the aqueous phase of the cytoplasm may play a role in redistributing cholesterol between different compartments of the cell. However, available data suggest that even if it happens, this is unlikely to play a significant physiological role.

1. Cholesterol in cells is compartmentalized with most of cholesterol residing in the plasma membrane (Liscum and Underwood, 1995). If passive diffusion of cholesterol is significant, such compartmentalization is unlikely to be preserved.

2. Transport of cholesterol from one compartment to another is rapid, unlike passive diffusion (Liscum and Dahl, 1992).

3. Steroid transport can be almost completely inhibited by pharmacological agents, and metabolic poisons which is not compatible with passive diffusion (Liscum and Dahl, 1992; Liscum and Underwood, 1995).

However, some data exist suggesting that passive diffusion cannot be dismissed from the range of possibilities for cholesterol trafficking. First, spontaneous transfer of cholesterol between cellular membranes *in vitro* is vectorial, occurs against a concentration gradient and is independent of the rate of cholesterol desorption from the donor membrane (Frolov et al., 1996b). This suggests that the apparent capacity of a membrane to accept cholesterol may not entirely depend on its cholesterol content, but rather on the overall organization of the membrane. Second, many pharmacological agents have a range of activities which can affect the structure of the membrane, for example its fluidity, thus affecting desorption of cholesterol or its lateral diffusion. Clearly, more studies are needed to evaluate (or dismiss) the contribution of this simplest form of trafficking to cholesterol homeostasis.

III. Overview of the general pathways of intracellular cholesterol trafficking

General pathways of cholesterol trafficking are found in all or many cell types and are not necessarily involved in serving a specialized function. These pathways are schematically presented at Fig. 1.

Entrance of exogenous cholesterol into cells is almost exclusively dependent on two subcellular structures. The first structure are the clathrin-coated pits. Lipoprotein particles (mainly LDL) bound to the high-affinity receptors are internalized and then deliver cholesteryl esters to the lysosomes by the classic endocytic pathway (see II.1). Cholesteryl esters are hydrolyzed in lysosomes to free cholesterol which is then delivered to the plasma membrane and endoplasmic

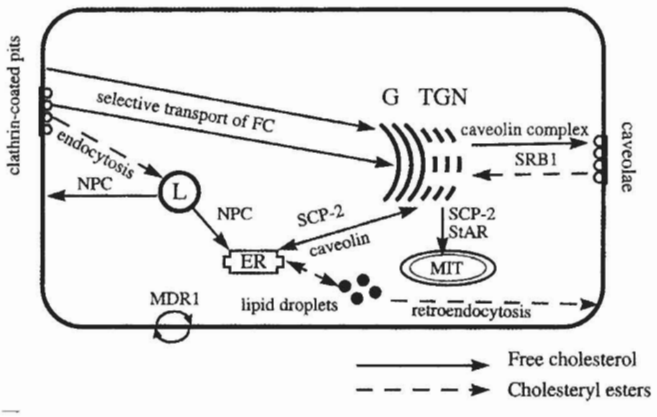

Fig. 1. Overview of the intracellular trafficking of cholesterol. ER: endoplasmic reticulum; G: Golgi apparatus; L: lysosome; MDR1: multidrug resistance protein 1; MIT: mitochondia; NPC: Niemann-Pick C Disease factor; SCP-2: sterol carrier protein 2; SR-B1: scavenger receptor type B1; StAR: steroidogenic acute regulatory protein; TGN: *trans*-Golgi network.

reticulum. The NPC gene is involved into this step of the pathway (see II. 5). It was estimated that about 70% of the cholesterol exiting lysosomes is destined for the PM with the remaining 30% delivered directly to the ER (Shamburek et al., 1997; Underwood et al., 1998). When delivered to the ER, exogenous cholesterol mixes with cholesterol from other sources forming a single pool of sterols. Other sources of cholesterol contributing to this pool are cholesterol synthesis and hydrolysis of stored cholesteryl esters. The ER therefore represents a dynamic mixing pool for exogenous and endogenous sterols. It must be noted however, that not all exogenous cholesterol enters the ER as some of LDL derived cholesterol is delivered to the TGN and the PM bypassing the ER. When there is an excess of sterols in the cell, cholesterol from the ER is esterified by ACAT and stored in the lipid droplets. Otherwise it is redistributed to the Golgi and *trans*-Golgi network, a process which is probably mediated by caveolin and assisted by SCP-2 (see II. 3 and II. 6). Another pathway connected to the clathrin-coated pits is selective uptake of free cholesterol from lipoproteins (mainly LDL) without internalization of the lipoprotein particle. This cholesterol is delivered directly to the TGN by a mechanism which is unknown.

The second point of entry involves SR-B1 (see II. 2) which is likely co-localized with caveolae (see II. 3). Cholesteryl esters are extracted from the lipoprotein particles (mainly HDL) bound to SR-B1 without internalization of the particle, hydrolyzed and free cholesterol is then delivered to the TGN (Reaven et al., 1996). The detailed mechanism of this pathway is also unknown.

At this point both endogenous and exogenous cholesterol become either esterified and moved for storage into lipid droplets, or are transferred to the TGN forming a pool of disposable free cholesterol. TGN therefore represents a sorting pool of sterols where the fate of cholesterol is decided. From the TGN cholesterol can be rapidly delivered to the PM, a pathway which includes caveolin complexes (II. 3 and II. 4). Free cholesterol from the TGN is specifically targeted to the caveolar region of the PM. Most cellular free cholesterol resides in the PM which therefore represents a repository pool of free cholesterol (Liscum and Faust, 1994). From the PM cholesterol can be: i) released to an extracellular cholesterol acceptor, like HDL or apoA-I (Fielding and Fielding, 1995c); ii) redistributed to other regions of the plasma membrane (Smart et al., 1996); iii) returned to the intracellular compartments (Lange et al., 1997), presumably to the TGN. The mechanism of the latter step is again unknown. As it is virtually impossible to completely block movement of cholesterol between TGN to the PM, it is likely that several pathways are contributing to the process. It seems clear however, that there is an effective and very fast transfer of cholesterol between a repository pool in the PM, a sorting pool in the TGN and a mixing pool in the ER.

When there is an excess of free cholesterol in the TGN and the PM some of it may be redistributed from the TGN back to the ER, where excess cholesterol is esterified by ACAT and stored in lipid droplets. The mechanism of cholesterol transfer from the TGN to the ER may involve caveolin and be assisted by SCP-2. Cholesteryl esters present in droplets may upon hydrolysis liberate free cholesterol which rejoins the circulation, or, at least in some cells, exit the cells via retroendocytosis. It is uncertain however if retroendocytosis is a general mechanism for the removal of cholesteryl esters. The mechanisms controlling cell cholesterol homeostasis are usually very effective and very little cholesterol needs to be stored as cholesteryl esters. Retroendocytosis may therefore be considered as part of a pathological condition associated with an excessive accumulation of cholesteryl esters.

Very little is known about regulation of intracellular cholesterol trafficking. A number of pharmacological agents inhibit some steps of cholesterol trafficking. Transport of cholesterol from the ER to the PM is inhibited by energy poisons and low temperature, but not by drugs affecting cytoskeleton (colchicine and cytochalasin B), inhibitor of protein biosynthesis (cycloheximide), ammonia, monensin and inhibitor of Golgi (brefeldin A) (Liscum and Dahl, 1992). Transport of cholesterol from lysosomes to the PM was inhibited by amines (U18666A, imipramine, stearylamine, RV-538) and sphinganine, but not by energy poisons, drugs affecting cytoskeletone (colchicine and cytochalasin B), ammonia, monensin, inhibitor of protein biosynthesis (cycloheximide) and protease inhibitor (leupeptin) (Liscum and Dahl, 1992). Okadaic acid and progesterone inhibited caveolin-dependent transport of cholesterol (Fielding and Fielding, 1995c). The only physiological regulator of cholesterol trafficking described so far is apoA-I which promotes translocation of cholesterol from the TGN to the PM (Slotte et al., 1987). This effect is likely to be mediated by protein kinase C (Mendez et al., 1991) and requires functional Golgi apparatus (and presumably TGN) (Mendez and Uint, 1996).

IV. Specialized pathways of intracellular cholesterol trafficking

In addition to the trafficking pathways described above, some cell types have specific functions which require specialized pathways of cholesterol trafficking. Some important examples of such pathways include: i) transport of dietary and biliary cholesterol from the brush border membrane of enterocytes to the ER during cholesterol absorption, ii) transport of cholesterol to sites of lipoprotein assembly in hepatocytes, enterocytes and macrophages, and iii) transfer of cholesterol to the mitochondria for the synthesis of steroid hormones in steroidogenic tissues.

Cholesterol absorption

Absorption of dietary and biliary cholesterol in the

gut is a complex pathway which involves trafficking of absorbed cholesterol within enterocytes (Wilson and Rudel, 1994). Cholesterol absorption is apparently a highly specific process since plant sterols and saturated cholesterol analogs are absorbed only fractionally to that of cholesterol (Heinemann et al., 1993; Sviridov et al., 1993; Wilson and Rudel, 1994). Intracellular events of the cholesterol absorption pathway include three steps: i) incorporation of cholesterol into the brush border membrane, ii) its transfer from the brush border to the ER, and iii) its esterification in the ER (Wilson and Rudel, 1994).

Incorporation of cholesterol into brush border membranes is most likely a passive, energy independent process (Westergaard and Dietschy, 1974), which does not discriminate between different sterols (Compassi et al., 1997). Therefore, cholesterol trafficking and/or cholesterol esterification most likely determine the specificity of cholesterol absorption. The only evidence in favor of cholesterol esterification determining the specificity of absorption is a much lower activity of ACAT toward plant sterols. The evidence supporting cholesterol trafficking to be a sterol-specific step include the following.

1. The rate of intracellular cholesterol trafficking is much higher compared with that of phytosterols (Sato et al., 1995).

2. Cholesterol, but not sitosterol absorption is tightly regulated, for example, in response to inhibition of cholesterol biosynthesis. This regulation is unlikely to affect cholesterol esterification (Sviridov et al., 1993; Safonova et al., 1994).

3. Sitosterol, stigmasterol and campesterol inhibit cholesterol trafficking from the PM to the ER without affecting ACAT activity toward cholesterol (Field et al., 1997), i.e. may act as antagonists.

4. Full inhibition of ACAT decreases cholesterol absorption, but does not bring it to the low level of sitosterol absorption (Sviridov et al., 1993).

The mechanism of dietary and biliary cholesterol transfer from the brush border membrane to the ER and the subsequent regulation of this process are unknown. However, since cholesterol absorption is inhibited when ACAT is inhibited (Sviridov et al., 1993; Marzetta et al., 1994; Wilson and Rudel, 1994; Sugiyama et al., 1995), yet is not accompanied by an increase in cell cholesterol content, the pathway is either bi-directional or is feedback regulated.

2. Lipoprotein assembly

Apolipoprotein B-containing lipoproteins are assembled in the liver (very low density lipoprotein, VLDL) and in the intestine (VLDL and chylomicrons). In both tissues the assembly of lipoprotein particles is initiated in the endoplasmic reticulum and finalized in the Golgi (Reaven and Reaven, 1977; Sabesin and Frase, 1977; Borchardt and Davis, 1987; Bostrom et al., 1988). Addition of lipids to apoB most likely occurs co-translationally, a step which plays an important role in the regulation of lipoprotein secretion (Innerarity et al., 1996). Another trafficking system which plays a key role in lipoprotein assembly, involves microsomal triglyceride transfer protein (MTP) which is essential for the assembly of apoB-containing lipoproteins in the liver and intestine (Sharp et al., 1993; Lin et al., 1994; Gordon et al., 1995; Wetterau et al., 1997). MTP is a heterodimer comprising of two subunits of 55 kDa and 97 kDa and resides on the ER (Gordon et al., 1995; Wetterau et al., 1997). In vitro MTP binds a range of lipids, but most effectively triglycerides and cholesteryl esters, and facilitates their transport between membranes (Jamil et al., 1995). *In vivo* deficiency of MTP leads to the inability of cells to assemble and secrete apoB-containing lipoproteins resulting in abetalipoproteinemia (Sharp et al., 1993). Transfection of HeLa cells with apoB and MTP resulted in initiation of lipoprotein assembly in these cells (Gordon et al., 1995). It was proposed that MTP is a *bona fide* lipid-binding protein, shuttling lipids from the ER membrane to the growing polypeptide chain of apoB. Co-translational delivery of lipids may be necessary to ensure correct folding of the polypeptide during its initial lipidation (Gordon et al., 1995). MTP is responsible for the transport of both triglycerides and cholesteryl esters, however, it is uncertain whether delivery of triglyceride or CE, or both, is the rate limiting step in the assembly of these lipoproteins. Several observations suggest that the availability of CE may be crucial (Field et al., 1987; Kam et al., 1989).

Apolipoprotein A-I containing lipoproteins most likely do not assemble intracellularly. Most apoA-I is secreted by hepatocytes and enterocytes in a lipid-free or lipid-poor form, and HDL particles are assembled by mobilization of lipids from either cell plasma membrane at the late stage or after secretion, or from circulating lipoproteins (Forte et al., 1993, 1995, 1996). Even if a small proportion of HDL is assembled intracellularly (N. Fidge, personal communication), availability of lipids is unlikely to be a rate-limiting step.

The third cell type capable of producing lipoproteins is the macrophage. Under normal conditions macrophages do not secrete lipoproteins, however, when loaded with cholesterol, discoid lipoprotein particles with apolipoprotein E as sole apolipoprotein are produced by macrophages (Kruth et al., 1994). It is not clear however, whether these lipoproteins are assembled inside the cell, like apoB-containing lipoproteins, or whether apoE, like apoA-I, is secreted in a lipid-poor form with subsequent uptake of cholesterol from the plasma membrane (Zhang et al., 1996).

3. Steroidogenesis

Steroid hormones are synthesized from cholesterol at the inner mitochondrial membrane of steroidogenic tissues. Cholesterol is not synthesized in the mitochondria and has to be delivered there. Cholesterol destined for steroid hormone synthesis, at least in rodents, originates from HDL-derived cholesteryl esters

internalized via SR-B1 (see above) and stored in lipid droplets in the TGN (Reaven et al., 1996). Initially SCP-2 was implicated in the trafficking of cholesterol to the mitochondria (Vahouny et al., 1987). However, recently another protein, Steroidogenic Acute Regulatory Protein (StAR) has been found to be essential for cholesterol trafficking to the mitochondria (Clark et al., 1994; Lin et al., 1995). StAR is a 285 amino acid protein with a mitochondrial targeting sequence of 25 residues on the amino-terminus, this sequence being cleaved off after transport into the mitochondria (Clark et al., 1994). Several patients with congenital lipoid adrenal hyperplasia, a disorder which is characterized by a deficiency of adrenal and gonadal steroid hormones, were found to have a mutation of StAR (Lin et al., 1995). Since the steroid hormone synthetic pathway was not affected in these patients, it was suggested that StAR was necessary for the transport of cholesterol to the site of the hormone synthesis (Lin et al., 1995). Transforming growth factor β_1, a potential physiological paracrine regulator of adrenocortical steroidogenesis, may repress StAR expression (Brand et al., 1998). The mechanism of transfer of cholesterol or cholesteryl esters from the TGN to the inner mitochondrial membrane by StAR is unknown. Hall and Alamahbobi (1997) have recently suggested another mechanism whereby intermediate filaments and acto-myosin facilitate physical contact between mitochondria and lipid droplets thus promoting direct transfer of cholesteryl esters from the latter to the former.

V. Concluding remarks

Information generated for the recent years clearly demonstrates the importance of intracellular cholesterol trafficking pathways in cholesterol metabolism, however, an understanding of the intimate mechanisms of the trafficking remains fragmentary. There is a number of reasons for that. Most of the initial data were obtained using pharmacological agents inhibiting cholesterol trafficking. These agents frequently have multiple activities making interpretation of results rather uncertain. Also, specific inhibition of pathways does not fully block the transport and only occasionally is a single mechanism exclusively responsible for a particular type of transport. However, in the case of genetic disorders, such as NPC, Zellweger syndrome (lack of SCP-2) or abetalipoproteinemia (lack of MTP), or genetic manipulations, such as transfection of caveolin or SR-B1 knockout, the contribution of certain pathways is clearer. All this ensures growing interest to the intracellular cholesterol trafficking and makes it likely that the picture will continue to be revised.

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