Chapter 8
Steroidogenic Acute Regulatory Protein-related Lipid Transfer (START) Proteins in Non-vesicular Cholesterol Transport

David B. Iaea, Shu Mao and Frederick R. Maxfield

Abstract Lipid transfer proteins play an important role in non-vesicular transport of sterols, phospholipids, and sphingolipids among intracellular membranes to maintain the proper sterol and lipid distribution. The steroidogenic acute regulatory protein-related lipid transfer (START) domain family are defined by a conserved 210 amino acid sequence that folds into an α/β helix-grip structure containing a hydrophobic pocket for ligand binding. The mammalian START proteins bind a variety of ligands, including cholesterol, phospholipids, sphingolipids, and bile acids with putative roles in non-vesicular lipid transport, tumor suppression, and thioesterase activity. However, the functions of many START proteins have yet to be well characterized. Recent studies have focused on determining the cell type distribution and expression profile of the START proteins, examining the ligand specificity and directionality of transport and characterizing disease states that may be associated with deregulation of START proteins. This chapter will summarize current findings regarding the physiologic and pathological roles of the START proteins in non-vesicular lipid transport.

Abbreviations

ACAT	acyl-CoA:cholesterol acyl-transferase
CHO	Chinese hamster ovary
DHE	dehydroergosterol
ER	endoplasmic reticulum
ERC	endocytic recycling compartment
EST	expressed sequence tag
HER2	human epidermal growth factor receptor 2
HMGR	HMG-CoA reductase
INSIG 1–2	insulin-induced genes 1 and 2
LDL	low density lipoproteins
LE/LY	late endosome and lysosomes
MENTAL	MLN64-NH2-terminal

F. R. Maxfield, Ph.D. (✉) · D. B. Iaea · S. Mao, Ph.D.
Department of Biochemistry, Weill Cornell Medical College,
1300 York Ave, New York, NY, 10065, USA
e-mail: frmaxfie@med.cornell.edu

B. J. Clark, D. M. Stocco (eds.), *Cholesterol Transporters of the START Domain Protein Family in Health and Disease,* DOI 10.1007/978-1-4939-1112-7_8,

MLN64	metastatic axillary lymph node 64 kDa protein
NPC	Niemann-Pick C
ORP	OSBP-related protein
OSBP	oxysterol binding protein
PC	phosphatidylcholine
PCTP	phosphatidylcholine transfer protein
PE	phosphatidylethanolamine
PH	pleckstrin homology
PM	plasma membrane
PS	phosphatidylserine
S1P and S2P	site 1 and site 2 proteases
SCAP	SREBP-cleavage-activating protein
SREBP-2	sterol regulatory element-binding protein-2
STARD	START-related domain
START	steroidogenic acute regulatory protein-related lipid transfer
VAP	vesicle-associated membrane protein-associated protein

Introduction

Our laboratory's initial interest was the basic cellular process of receptor-mediated endocytosis [1], and our main research tool has been quantitative fluorescence microscopy. We initially used low-density lipoproteins (LDL) as a fluorescent probe to study endocytic pathways because they could be labeled brightly, which was very useful given the cameras available in the 1980s and 1990s. Later, in collaboration with Ira Tabas at Columbia University, we began to explore the role of lipoproteins in the formation of macrophage foam cells [2]. This led to investigations into how LDL-derived cholesterol gets from late endosomes and lysosomes (LE/LY) into the endoplasmic reticulum (ER; [3]), followed by a realization that this process was not well understood. We then explored the use of a fluorescent sterol, dehydroergosterol (DHE), that had been studied extensively by Schroeder's group [4, 5], and we began to study its intracellular transport [6]. It became obvious that there was extensive non-vesicular intracellular transport of sterols in mammalian cells, and we wanted to understand what the carriers might be. Jan Breslow, at Rockefeller University, discussed STARD4 with us because of his interest in this as an SREBP2-regulated protein, and we began a collaboration to investigate the possible role of STARD4 and STARD5 in sterol transport. Our initial studies were not successful, mainly because the microscopy tools available at the time were inadequate. With better instruments and a better understanding of the cell biology, we were able to publish two papers to date on the cellular role of STARD4 in sterol transport and regulation [7, 8]. We continue to explore this fascinating but challenging family of proteins.

Sterols are ubiquitous components of cell membranes in eukaryotes. Among the major lipid components of eukaryotic membranes, sterols have a unique chemical

structure. Cholesterol, a 27 carbon molecule, contains a single hydroxyl as the only polar component, four planar rings and a short alkyl chain [9]. Its structure is in contrast with most phospholipids, which are composed of large polar headgroups and long hydrocarbon chains. Cholesterol is heterogeneously distributed among cellular organelles with ~60 % of total cellular cholesterol in the plasma membrane (PM) of mammalian cells, while relatively low amounts are maintained in the mitochondria and ER, the latter being the site of cholesterol biosynthesis and storage [10–14]. In several cell types, the endocytic recycling compartment (ERC) has been shown to be a major pool of intracellular cholesterol [6, 15].

Cellular cholesterol content has major effects on the biophysical properties of membranes [16], which can alter the functional properties of membrane proteins [17, 18]. Therefore, maintaining the proper distribution of cholesterol among cellular membranes is required for homeostasis. As discussed in this chapter, sterol transport among organelles plays a key role in the cell's mechanisms for maintaining cholesterol homeostasis. At the cellular level, excess cholesterol can be regulated rapidly by esterification by acyl-CoA:cholesterol acyl-transferase (ACAT) followed by storage in lipid droplets. The rate-limiting step in esterification of cholesterol by ACAT is delivery of cholesterol to the ER, which can be assisted by various lipid transfer proteins [15, 19]. At the level of the whole organism, cholesterol is metabolized in the liver to bile acids, and excretion of bile is the major pathway for elimination of cholesterol. Dysregulation of cholesterol homeostasis is an important contributor to diseases such as atherosclerosis and some lysosomal storage disorders, and it can play a role in some cancers as well [20–22].

Cellular cholesterol levels are tightly regulated by coordinated biosynthetic and efflux pathways. Mammalian cells obtain cholesterol by two primary mechanisms: uptake from lipoproteins and *de novo* biosynthesis. Figure 8.1 illustrates pathways of intracellular sterol transport. Alterations in the cholesterol content of the ER causes profound changes in the expression of genes involved in the biosynthesis and uptake of cholesterol through the actions of the Insig-SCAP-SREBP2 proteins [23]. Endogenous cellular cholesterol biosynthesis occurs in the ER, which contains the major rate-limiting enzyme in cholesterol biosynthesis, HMG-CoA reductase (HMGR). HMGR expression is transcriptionally regulated by the sterol regulatory element-binding protein-2 (SREBP2) transcription factor [24]. When cellular sterols are abundant, SREBPs are retained in the ER membrane in a complex with the cholesterol-sensing protein, SREBP-cleavage-activating protein (SCAP), and the ER retention proteins insulin-induced genes 1 and 2 (INSIG 1–2) [11].

When cholesterol content in the ER is depleted, SCAP undergoes a conformational change and is released from Insig, allowing the SREBP2-SCAP complex to translocate to the Golgi apparatus [13]. In the Golgi, SREBP2 is proteolytically processed by the site 1 and site 2 proteases (S1P and S2P) to release the 50 kDa amino (N)-terminal transcription factor that translocates to the nucleus to activate the expression of genes involved in sterol biosynthesis, uptake, and metabolism [25]. Therefore, changes in cholesterol levels in membranes such as the PM must

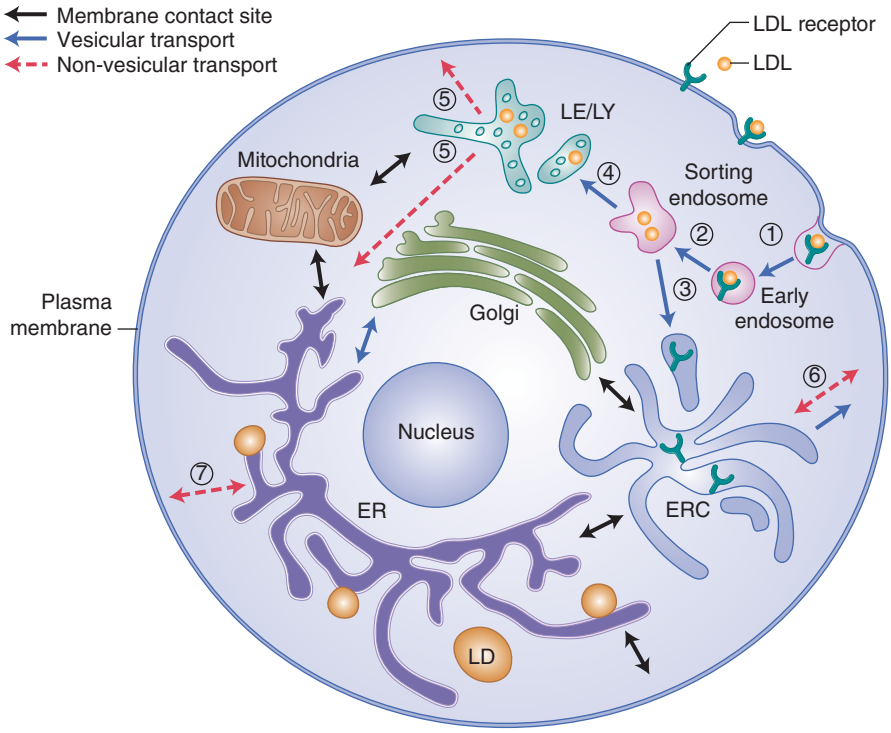

Fig. 8.1 1, 2 LDL particles containing cholesterol-esters and free cholesterol are internalized by clathrin-mediated endocytosis of the LDL receptor [1]. 3 The low pH in early endosomes causes the LDL to be released from its receptor, and the empty LDL receptor returns to the cell surface for further rounds of endocytosis. 4 The LDL is retained in the early sorting endosomes, which mature into late endosomes where it encounters acid hydrolases including lysosomal acid lipase, which hydrolyzes the cholesteryl esters in the core of the LDL particles. 5 The free cholesterol is transported out of LE/LY and delivered to other cellular membranes including the plasma membrane and ER by primarily non-vesicular mechanisms. 6 Cholesterol in the plasma membrane can traffic to the ERC and back by both vesicular and non-vesicular mechanisms. 7 Cholesterol transport from the plasma membrane to the ER informs the homeostatic machinery about the free cholesterol levels in the cell. *LDL* low density lipoproteins, *LE/LY* late endosome and lysosomes, *ER* endoplasmic reticulum, *ERC* endocytic recycling compartment

be sensed in the ER to maintain cholesterol homeostasis. Studies of lipid trafficking from the PM generally find extremely low levels of delivery to the ER [26], indicating that non-vesicular transport must be required for effective movement of cholesterol from the PM [19] to the ER [1].

This poses an interesting question in that, in order for cholesterol levels sensed in the ER to reflect the cholesterol distribution and content in other organelles, such as the PM and endosomes, there must be a mechanism for rapid redistribution of cholesterol. Several lines of evidence indicate that non-vesicular transport plays an important role in maintaining the correct distribution of cholesterol among organ-

elles. Lipid transfer proteins play an important role in non-vesicular transport of sterols and lipids among intracellular organelles [27]. They also play an important role in sensing abundance of lipids and regulating cellular physiology [28]. Newly synthesized cholesterol in the ER is rapidly trafficked to the PM, by non-vesicular mechanisms [29–31]. A fluorescent sterol, DHE, showed a distribution in Chinese hamster ovary (CHO) fibroblasts similar to cholesterol [6]. When the DHE in the ERC was photobleached, much of it was replaced by DHE from elsewhere in the cell (mainly the PM) within a few minutes [6]. Most of this transport of DHE was adenosine triphosphate (ATP)-independent, so it must have been by a non-vesicular process. Additionally, following receptor mediated endocytosis of LDL, free cholesterol is generated by hydrolysis of cholesterol esters in the LE/LY compartments [32]. The cholesterol that is released from the LE/LY is distributed throughout the cell, including the PM [19] and the ER [1, 33]. Since there are not major vesicular trafficking routes from the LE/LY to either the PM or the ER, it is likely that much of this transport is non-vesicular.

There are several protein families that are classified as sterol transfer proteins, which have been shown to be capable of transferring sterols between membranes [34]. Sterol transfer proteins can interact directly with membrane compartments to extract sterol and then diffuse in complex with the sterol to acceptor membranes [35]. In some cases, such as ER-PM contact sites, the membranes of two organelles are held in close proximity by protein complexes, and this can facilitate rapid exchange of sterol between the membranes by reducing the distance that the sterol:protein complex must travel. This mechanism would also provide a means for targeted delivery of sterol from donor to acceptor membranes. Two major gene families for lipid transfer proteins that have been implicated in such trafficking are the steroidogenic acute regulatory protein (StAR)-related lipid-transfer (START) domain family [36] and the oxysterol-binding protein (OSBP) and OSBP-related protein (ORP) family, which have been discussed in detail elsewhere [37, 38]. This chapter will summarize the current knowledge of the physiologic roles of the START proteins in non-vesicular lipid transport.

Sterol Transport Proteins and the START Domain Family

The START domain is defined by a 210 amino acid sequence that folds into an α/β helix-grip structure containing a hydrophobic pocket for ligand binding [39, 40]. Database analyses have identified START domains in the genomes of plants, bacteria, protists, and animals, but they have not been identified in either archaea or yeast. Interestingly, START domains are most abundant in plants and are found in proteins that contain a homeodomain, suggesting a role in transcriptional regulation [41]. This unique protein architecture of the START-homeodomain is found only in plants. However, the presence of a START domain in conjunction with other motifs, to form multi-domain proteins, is common in various phyla and permits additional

protein functionality such as enzymatic activity, signaling, and protein localization [42].

The mammalian START domain protein family is composed of 15 members that group into six subfamilies based on domain architecture and ligand specificity [42]. In general terms, there are the cholesterol/oxysterol binding proteins (STARD1/3 subfamily), soluble proteins (STARD4/5/6 subfamily), phospholipid- and sphingolipid-binding proteins (STARD2 [phosphatidylcholine transfer protein, PCTP]/7/8/10/11 subfamily), putative Rho-GTPase signaling (STARD8/12/13 subfamily), thioesterase activity containing proteins (STAR4/15 subfamily), and the STARD9 subfamily composed of a single member.

Mechanism of START-mediated Sterol Transport

The crystal structures of human STARD3/metastatic axillary lymph node 64 kDa protein (MLN64) and murine STARD4 were among the first of the START proteins to be described [39, 43]. The structures of these proteins showed an α/β helix-grip structure composed of nine anti-parallel β sheets flanked at the amino- and carboxyl-termini by α helices. The core of this protein forms a hydrophobic pocket that would accommodate a single sterol molecule. The suggested mechanisms for START domain cholesterol transfer has been described elsewhere [44, 45]. In brief, structural and biophysical studies have proposed two models: Movement of the carboxyl termini helix leads to a molten globule transition to facilitate cholesterol absorption and release [44, 46]. Alternatively, molecular dynamics studies have proposed that movement of the omega-1 loop, following membrane binding, may be sufficient for activity [45]. Comparing the structure of several sterol transfer proteins, demonstrates that they each contain a hydrophobic ligand pocket capable of binding a single sterol molecule [43, 47, 48]. In several of these proteins, the sterol-binding pocket is under “gated” regulation that could open and close upon interaction with the lipid bilayer, as suggested by molecular dynamic studies, to facilitate sterol/lipid absorption and delivery to specific membranes. To date, several crystal structures of START family members have been reported, confirming that the helix-grip structure is maintained across five mammalian START subfamilies [49, 50].

The Soluble Sterol Transporters STARD4 Subfamily

The STARD4 subfamily is composed of STARD4, STARD5, and STARD6. This subfamily is most closely related to the STARD1/3 subfamily with approximately 25% sequence identity in the START domains [51]. STARD4 was identified as a novel expressed sequence tag (EST) in a microarray designed to identify genes that are transcriptionally regulated by cholesterol in the liver. Briefly, mice that were fed a high cholesterol diet had reduced STARD4 messenger ribonucleic acid

(mRNA) levels in comparison to control animals. Expression of STARD4 in 3T3-L1 fibroblasts and a human monocytic cell line derived from an acute monocytic leukemia patient (THP-1) was suppressed by sterol overloading and enhanced by statin treatment [52]. These findings are consistent with STARD4 being transcriptionally regulated by cellular cholesterol levels via SREBP-2 [53]. Subsequently, STARD5 and STARD6 were identified by BLAST search of the whole genome and EST databases [51]. Analysis of the protein-coding sequence for the STARD4 family indicates a protein of ~25 kDa that is entirely composed of the START domain without a membrane targeting sequence. The cytosolic distribution of these START proteins has led to increased interest and speculation regarding the role of these proteins in cholesterol trafficking and homeostasis. To understand the role of this START protein subfamily, we will discuss the properties and proposed functions of its members.

STARD4: Cholesterol Trafficking Among Intracellular Organelles

STARD4 is widely expressed in a variety of tissues [51] and was detected by immunohistochemistry in human hepatocytes and Kupffer cells [52]. The subcellular localization of STARD4 is difficult to ascertain. As a soluble cytoplasmic protein with no obvious targeting motif, it may be distributed throughout the cytoplasm. Immunolocalization requires permeabilization of cells with detergents, which can release many cytoplasmic proteins even in fixed cells and can also disrupt the membranes to which a protein such as STARD4 may bind. It has been reported that STARD4 can be seen associated with the ER by immunolocalization [52], but the fraction that is associated with the ER in the intact cell is difficult to determine.

Several lines of evidence, both in cells and in vitro, show that STARD4 can transfer cholesterol efficiently. STARD4 can transfer sterol between liposomes in a process that depends on the composition of the donor and acceptor membranes [7]. Analysis of the STARD4 surface revealed a region enriched in basic residues near the sterol-binding pocket that facilitates STARD4's interaction with negatively charged membranes [7]. Mutations in the basic patch markedly decreased the sterol transfer rate of STARD4, indicating that the interaction with anionic lipids is required for maximal activity. This finding is of particular interest since the cytoplasmic leaflets of the PM, the ERC, and ER are highly enriched in anionic lipids, particularly phosphatidylserine [54]. Additionally, the sterol transfer rate of STARD4 was increased by threefold when acceptor liposomes were enriched in unsaturated lipids [7]. Since the ER is more highly enriched in unsaturated lipids than other organelles [55], STARD4-mediated delivery of sterol to the ER could be enhanced by the abundance of unsaturated lipids.

Several experimental results support the hypothesis that STARD4 plays an important role in delivery of cholesterol to the ER. In studies with isolated microsomes, cholesterol esterification by ACAT1, an ER enzyme, is stimulated by purified STARD4 [52]. Overexpression of STARD4 in mouse hepatocytes leads to an increase in cholesterol esterification by ACAT [52]. The role of STARD4 in

transporting cholesterol to the ER is further supported by a recent study of STARD4 in U2OS human osteosarcoma cells [7]. STARD4 overexpression increased cholesterol ester levels. STARD4 silencing by small interfering RNA (siRNA) attenuated cholesterol-mediated regulation of SREBP-2 activation, while STARD4 overexpression amplified sterol sensing by the SCAP/SREBP-2 proteins in the ER. Silencing STARD4 increased the cellular cholesterol levels, presumably as a result of the slower delivery of cholesterol to the ER. Interestingly, this effect of reduced STARD4 expression on cholesterol levels could be reversed by microinjection of methyl-β-cyclodextrin, a cyclic sugar that nonselectively exchanges cholesterol among membranes. These data suggest that delivery of cholesterol to the sterol-sensing membranes of the ER is an important function of STARD4.

STARD4 also plays a role in sterol transport to other organelles. When U2OS cells are incubated with the fluorescent sterol, DHE, the ERC is the major intracellular organelle that is labeled [6]. Fluorescence recovery after photobleaching the ERC allows measurement of the rate of transport of DHE back into the ERC, and overexpression of STARD4 significantly increased the transport of DHE into the ERC [7]. Knockdown of STARD4 in HepG2 cells resulted in decreased sterol transport to the ERC as well as reduced ER-associated cholesterol and cholesterol esters [8]. Again, microinjection of methyl-β-cyclodextrin into the cells had a similar effect to overexpression of STARD4. Transient expression of STARD4 in Cos-1 cells resulted in an increase in steroidogenesis though at a lower efficiency than StAR and STARD3 [53, 56], suggesting that STARD4 may be able to deliver cholesterol to mitochondria. This indicates that there may be some overlap in the function of the StAR sterol transporters.

Increased cholesterol transport to the ER may result in ER stress. While it is well established that STARD4 is transcriptionally regulated by SREBP-2, STARD4 mRNA was also found to be increased following transient treatment with tunicamycin, a small molecule activator of ER stress [53, 57]. While the functional relevance of increased STARD4 mRNA following ER stress is unknown, transient increases in STARD4 under disease conditions associated with ER stress may affect cholesterol homeostasis by increasing cholesterol delivery to the ER, which would increase both SREBP-2 processing and cholesterol esterification.

Recently, a homozygous STARD4 knockout mouse has been described [58]. Interestingly, STARD4 knockout mice develop normally and do not present with a distinct lipid phenotype; plasma and hepatic lipid profiles of STARD4 knockout mice were similar to wild-type mice. Female STARD4 knockout mice did have decreased phospholipid and cholesterol content in their gallbladder [58]. Statin treatment did not alter the hepatic or plasma lipid profiles of wild-type or STARD4 knockout mice. The mRNA levels of various START proteins were not significantly altered in STARD4 knockout mice, indicating that expression of other START proteins is not responsive to loss of STARD4 [58]. This likely indicates that there is redundancy for sterol transport, beyond the START family, in mammalian cells. It remains unclear whether there are stresses—dietary or disease specific—that may be exacerbated by loss of STARD4.

STARD5 and ER Stress

STARD5 has been reported to bind cholesterol as well as 25-hydroxycholesterol, and it is widely expressed in a variety of tissues with the highest expression in liver and kidney [51, 59]. In the liver, STARD5 is localized solely to the Kupffer cells [60]. STARD5 does not transfer cholesterol to mitochondria in cells or in vitro [61]. Expression of STARD5 in hepatocytes results in a threefold increase in free cholesterol levels, particularly in the Golgi region and possibly the ER [60]. As a sterol transporter, it has been suggested that STARD5 may shuttle cholesterol among organelles [60, 62]. However, recent structural studies of STARD5 have shown that it does not bind cholesterol but rather cholic acid, a precursor in bile acid biogenesis [63]. With this new finding, further studies will be required to identify the biologically relevant substrates of STARD5. It is reported that unlike STARD4, STARD5 overexpression does not result in an increase in cholesterol ester formation, but increases free cholesterol levels, indicating distinct roles and functions for these sterol transporters in cholesterol homeostasis [7, 59, 61]. Additionally, overexpression of STARD5 in macrophages increased SREBP2 mRNA levels, which may result in increase in cholesterol biosynthesis [64]. It is likely that STARD5 participates in some aspect of cholesterol regulation, but its precise role is uncertain.

STARD5 mRNA levels are increased following treatment with agents that promote ER stress, such as thapsigargin in 3T3 cells or cholesterol overloading in J774 macrophages [53, 62]. The precise function of STARD5 under ER stress is not known, but chronic ER stress is associated with several metabolic disease states including type 2 diabetes and cancer [62, 65]. In a diabetic mouse model, STARD5 mRNA and protein steady state levels were significantly increased, and free cholesterol levels were elevated compared to control animals [62].

STARD6: Cholesterol Transport to the Mitochondria

STARD6 was identified as being predominantly expressed in testes and was later shown to be solely localized to the germ lines, with highest expression in spermatids [51]. However, the function of STARD6 in spermatids remains unknown. Recent work has suggested a role for STARD6 in sperm motility and quality as it is a gene required for mitochondrial nicotinamide adenine dinucleotide hydrogen (NADH)-dependent dehydrogenase activity [66]. This finding is in agreement with previous reports that STARD6 transports cholesterol to the mitochondria as effectively as StAR and STARD3 [46, 61]. These findings suggest that STARD6 may function to deliver cholesterol to the mitochondria in male germ cells, but the exact functions remains to be determined. Like the other members of the STARD4 subfamily, STARD6 lacks an organelle-targeting sequence, so further work is required to investigate the role of STARD6 in cholesterol transport to specific organelles (mitochondria, ER, or PM) in male germ cells [42].

The Oxysterol-Cholesterol-Binding Proteins STARD1/3

StAR and STARD3 are similar in that both START domains are selective for cholesterol and are targeted to specific membrane compartments [67]. StAR is the prototypic and founding member of the START domain protein family. StAR is primarily expressed in the adrenal and gonads. Among the START domain family, StAR contains a classical amino-terminal-targeting sequences that directs it to the mitochondria [68]. Recent biochemical studies indicate that StAR may form a functional complex with a cholesterol transfer channel where it facilitates cholesterol transport from the outer to inner mitochondrial membrane [69, 70]. However, the precise molecular mechanism for StAR cholesterol transfer across the membranes of mitochondria is not known although various studies have provided insights into this process. Greater details regarding the current model of StAR and its mechanism of action can be found in Chaps. 2 and 3 as well as recent reviews [36, 44, 68].

STARD3 and Cholesterol Efflux from LE/LY

STARD3/MLN64 is a multi-pass transmembrane protein with an MLN64-NH2-terminal (MENTAL) domain that localizes to late endosomes with the START domain orientated into the cytosol at the carboxy terminus [71, 72]. The localization of MLN64 to late endosomes has suggested a role in cholesterol efflux from this compartment as well as a potential role in Niemann-Pick C (NPC) disease, which has been discussed in preceding chapters [27, 73]. NPC disease is a lipid storage disorder caused by mutation in either NPC1 or NPC2 that results in accumulation of free cholesterol in LE/LY, leading to systemic and neurological disorders [20]. Briefly, following endocytosis of LDL and generation of free cholesterol by lysosomal acid lipase in LE/LY, NPC2 binds cholesterol and transfers it to the cholesterol-binding domain of NPC1, a transmembrane protein in the limiting membrane of the lysosome. NPC1 transfers cholesterol out of the lysosome by an unknown mechanism [74, 75]. STARD3 has been proposed to participate in the efflux of cholesterol from the LE/LY, but the mechanism for this is unclear [73]. It has been proposed that STARD3 plays a role in cholesterol transport from the LE/LY to mitochondria for steroidogenesis [75]. However, the exact function of STARD3/MLN64 in cholesterol trafficking remains unclear [76].

The Phospho/Sphingolipid-Binding START Proteins

This subfamily of the START protein is composed of the phosphatidylcholine (PC)-binding STARD2 (PCTP), STARD7, and STARD10 as well as STARD11, which binds ceramide [42]. Of the STARD2 family, STARD2/PCTP and STARD11/CERT have been extensively studied and reviewed [50, 77, 78]. Purified PCTP was shown

to selectively transport PC between membranes in cells and in vitro [79, 80]. A potential function for STARD2 is to facilitate PC delivery to the hepatic canalicular and alveolar membrane for incorporation into bile acids and lung surfactant synthesis, respectively [81]. The crystal structure of PCTP bound to PC demonstrates the characteristic helix-grip fold and hydrophobic tunnel of the START family. Interestingly, the PC head group provides ligand specificity, and the hydrophobic tunnel can accommodate PC regardless of acyl chain saturation [49].

STARD7 was originally identified as a transcript overexpressed in placental choriocarcinoma cells [82]. It shares ~25% sequence identity with PCTP and selectively transfers PC but not phosphatidylserine (PS], phosphatidylethanolamine (PE), or sphingomyelin between membranes in vitro [83]. Similarly, STARD10 was identified as a phosphoprotein overexpressed in tumors of the human epidermal growth factor receptor 2 (HER2) transgenic mouse [84]. Purified STARD10 transfers PC and PE in vitro and in vivo but displays some ligand specificity toward PC in comparison to PE [85].

STARD7 and PC Distribution

A variant of STARD7, STARD7–1, was identified by protein database analysis and was found to have an extended amino terminus that forms an amphipathic helix to localize the protein to the mitochondria [83]. In a mouse hepatoma cell line, overexpression of STARD7 resulted in an increase in mitochondrial PC levels while silencing using siRNA did not alter PC levels in the mitochondria. STARD7 may function at the cytoplasmic leaflet of the outer mitochondrial membrane to regulate mitochondrial PC levels [83]. STARD7 is highly expressed in proliferating cells including lung, colon, and liver cells and may function to deliver PC for mitochondrial biogenesis, function, and homeostasis.

STARD11/CERT and Ceramide Transport

STARD11, more commonly known as ceramide transfer protein (CERT), is a ceramide transport protein that acts to transport ceramide from the ER to the Golgi [86], where it is converted to sphingomyelin and glucosylceramide [87]. Unlike the other members of the STARD2 family, STARD11/CERT has additional structural domains that target the START domain to selective membrane compartments for cellular function. CERT has an amino terminal pleckstrin homology (PH) domain, a central Two phenylalanines in an acidic tract (FFAT) motif, and a carboxy terminal START domain. The PH domain and the FFAT motif act to target CERT to either the Golgi by binding of the PH domain to the phosphoinositide, PI4P, or to the ER by interaction with the ER resident protein vesicle-associated membrane protein-associated protein (VAP), via the FFAT motif [72]. The phosphorylation of CERT is proposed to enhance membrane interaction by allowing the PH and FFAT motifs

to interact with their respective membrane targets in order to position the START domain for ceramide extraction and delivery [78, 86]. The structure of the START domain of CERT has been determined and confirms the helix-grip fold and hydrophobic pocket that would facilitate ceramide binding [50].

Summary

As illustrated in Fig. 8.1, non-vesicular lipid trafficking plays an essential role in the maintenance of cellular lipid distribution. Recent advances in biochemistry and cell biology have led to the description of several basic properties, including the rate of sterol transport. Unfortunately, very little is understood about the molecular mechanisms involved in non-vesicular transport. Several proteins have been identified as sterol transporters including the START and ORP families. Of the mammalian START domain proteins, the STARD1/3 and STARD4 families are involved in sterol transport. While the biological function of StAR is well established, further work remains to elucidate the functional relevance of STARD3/4/5/6. Interestingly, STARD4 and STARD5 have been proposed to function at the ER although with distinct roles. Knockout animals of STARD4 were viable and did not have altered lipid profiles. However, it is possible that a phenotype following loss of STARD4 could potentially be exacerbated by dietary or disease related stresses. The STARD2/PCTP subfamily functions in a variety of pathways and transports several different lipids. It is important to continue to examine START protein expression and function in both normal and disease states in order to establish and distinguish their biological significance in lipid homeostasis.

Acknowledgements Work in the Maxfield laboratory is supported by National Institutes of Health (NIH) grant R37-DK27083.

References

1. Mukherjee S, Ghosh RN, Maxfield FR. Endocytosis. Physiol Rev. 1997;77(3):759–803.
2. Myers JN, Tabas I, Jones NL, Maxfield FR. Beta-very low density lipoprotein is sequestered in surface-connected tubules in mouse peritoneal macrophages. J Cell Biol. 1993;123(6 Pt 1):1389–402.
3. Skiba PJ, Zha X, Maxfield FR, Schissel SL, Tabas I. The distal pathway of lipoprotein-induced cholesterol esterification, but not sphingomyelinase-induced cholesterol esterification, is energy-dependent. J Biol Chem. 1996;271(23):13392–400.
4. McIntosh AL, Atshaves BP, Huang H, Gallegos AM, Kier AB, Schroeder F. Fluorescence techniques using dehydroergosterol to study cholesterol trafficking. Lipids. 2008;43(12):1185–208.
5. Schroeder F, Barenholz Y, Gratton E, Thompson TE. A fluorescence study of dehydroergosterol in phosphatidylcholine bilayer vesicles. Biochemistry. 1987;26(9):2441–8.
6. Hao M, Lin SX, Karylowski OJ, Wustner D, McGraw TE, Maxfield FR. Vesicular and non-vesicular sterol transport in living cells. The endocytic recycling compartment is a major sterol storage organelle. J Biol Chem. 2002;277(1):609–17.

7. Mesmin B, Pipalia NH, Lund FW, Ramlall TF, Sokolov A, Eliezer D, et al. STARD4 abundance regulates sterol transport and sensing. Mol Biol Cell. 2011;22(21):4004–15.
8. Garbarino J, Pan M, Chin HF, Lund FW, Maxfield FR, Breslow JL. STARD4 knockdown in HepG2 cells disrupts cholesterol trafficking associated with the plasma membrane, ER, and ERC. J Lipid Res. 2012;53(12):2716–25.
9. van Meer G. Cellular lipidomics. EMBO J. 2005;24(18):3159–65.
10. Ikonen E. Cellular cholesterol trafficking and compartmentalization. Nat Rev Mol Cell Biol. 2008;9(2):125–38.
11. Radhakrishnan A, Goldstein JL, McDonald JG, Brown MS. Switch-like control of SREBP-2 transport triggered by small changes in ER cholesterol: a delicate balance. Cell Metab. 2008;8(6):512–21.
12. Maxfield FR, Menon AK. Intracellular sterol transport and distribution. Curr Opin Cell Biol. 2006;18(4):379–85.
13. Blanchette-Mackie EJ, Pentchev P. Cholesterol distribution in Golgi, lysosomes and endoplasmic reticulum. In: Chang TY, Freeman D, editors. Intracellular cholesterol trafficking. US: Springer; 1998. S. 53–73.
14. Mobius W, van Donselaar E, Ohno-Iwashita Y, Shimada Y, Heijnen HFG, Slot JW, et al. Recycling compartments and the internal vesicles of multivesicular bodies harbor most of the cholesterol found in the endocytic pathway. Traffic. 2003;4(4):222–31.
15. Liscum L, Munn NJ. Intracellular cholesterol transport. Biochim Biophys Acta. 1999;1438(1):19–37.
16. Huang J, Feigenson GW. A microscopic interaction model of maximum solubility of cholesterol in lipid bilayers. Biophys J. 1999;76(4):2142–57.
17. Pani B, Ong HL, Liu X, Rauser K, Ambudkar IS, Singh BB. Lipid rafts determine clustering of STIM1 in endoplasmic reticulum-plasma membrane junctions and regulation of store-operated Ca2+ entry (SOCE). J Biol Chem. 2008;283(25):17333–40.
18. Galan C, Woodard GE, Dionisio N, Salido GM, Rosado JA. Lipid rafts modulate the activation but not the maintenance of store-operated Ca(2+) entry. Biochim Biophys Acta. 2010;1803(9):1083–93.
19. Das A, Goldstein JL, Anderson DD, Brown MS, Radhakrishnan A. Use of mutant 125I-Perfringolysin O to probe transport and organization of cholesterol in membranes of animal cells. Proc Natl Acad Sci U S A. 2013;110(26):10580–5.
20. Rosenbaum AI, Maxfield FR. Niemann-Pick type C disease: molecular mechanisms and potential therapeutic approaches. J Neurochem. 2011;116(5):789–95.
21. Siperstein MD, Fagan VM. Deletion of the cholesterol-negative feedback system in liver tumors. Cancer Res. 1964;24:1108–15.
22. Tabas I. Apoptosis and plaque destabilization in atherosclerosis: the role of macrophage apoptosis induced by cholesterol. Cell Death Differ. 2004;11(Suppl 1):2–6.
23. Goldstein JL, DeBose-Boyd RA, Brown MS. Protein sensors for membrane sterols. Cell. 2006;124(1):35–46.
24. Brown MS, Goldstein JL. Cholesterol feedback: from Schoenheimer's bottle to Scap's MELADL. J Lipid Res. 2009;50(Suppl):5–27.
25. Horton JD, Shah NA, Warrington JA, Anderson NN, Park SW, Brown MS, et al. Combined analysis of oligonucleotide microarray data from transgenic and knockout mice identifies direct SREBP target genes. Proc Natl Acad Sci U S A. 2003;100(21):12027–32.
26. Wustner D, Mondal M, Tabas I, Maxfield FR. Direct observation of rapid internalization and intracellular transport of sterol by macrophage foam cells. Traffic. 2005;6(5):396–412.
27. Mesmin B, Maxfield FR. Intracellular sterol dynamics. Biochim Biophys Acta. 2009;1791(7):636–45.
28. Li X, Rivas MP, Fang M, Marchena J, Mehrotra B, Chaudhary A, et al. Analysis of oxysterol binding protein homologue Kes1p function in regulation of Sec14p-dependent protein transport from the yeast golgi complex. J Cell Biol. 2002;157(1):63–77.
29. Maxfield FR, van Meer G. Cholesterol, the central lipid of mammalian cells. Curr Opin Cell Biol. 2010;22(4):422–9.

30. Urbani L, Simoni RD. Cholesterol and vesicular stomatitis virus G protein take separate routes from the endoplasmic reticulum to the plasma membrane. J Biol Chem. 1990;265(4):1919–23.
31. Baumann NA, Sullivan DP, Ohvo-Rekila H, Simonot C, Pottekat A, Klaassen Z, et al. Transport of newly synthesized sterol to the sterol-enriched plasma membrane occurs via nonvesicular equilibration. Biochemistry. 2005;44(15):5816–26.
32. Goldstein JL, Brown MS. The LDL receptor. Arterioscler Thromb Vasc Biol. 2009;29(4):431–8.
33. Maxfield FR, McGraw TE. Endocytic recycling. Nat Rev Mol Cell Biol. 2004;5(2):121–32.
34. D'Angelo G, Vicinanza M, De Matteis MA. Lipid-transfer proteins in biosynthetic pathways. Curr Opin Cell Biol. 2008;20(4):360–70.
35. Mesmin B, Antonny B, Drin G. Insights into the mechanisms of sterol transport between organelles. Cell Mol Life Sci. 2013 Sept;70(18):3405–21.
36. Clark BJ. The mammalian START domain protein family in lipid transport in health and disease. J Endocrinol. 2012;212(3):257–75.
37. Prinz WA. Non-vesicular sterol transport in cells. Prog Lipid Res. 2007;46(6):297–314.
38. Beh CT, McMaster CR, Kozminski KG, Menon AK. A detour for yeast oxysterol binding proteins. J Biol Chem. 2012;287(14):11481–8.
39. Tsujishita Y, Hurley JH. Structure and lipid transport mechanism of a StAR-related domain. Nat Struct Biol. 2000;7(5):408–14.
40. Iyer LM, Koonin EV, Aravind L. Adaptations of the helix-grip fold for ligand binding and catalysis in the START domain superfamily. Proteins-Struct Funct Genet. 2001;43(2):134–44.
41. Ponting CP, Aravind L. START: a lipid binding domain in StAR, HD-ZIP and signalling proteins. Trends Biochem Sci. 1999;24(4):130–2.
42. Soccio RE, Breslow JL. StAR-related lipid transfer (START) proteins: mediators of intracellular lipid metabolism. J Biol Chem. 2003;278(25):22183–6.
43. Romanowski MJ, Soccio RE, Breslow JL, Burley SK. Crystal structure of the *Mus musculus* cholesterol-regulated START protein 4 (StarD4) containing a StAR-related lipid transfer domain. Proc Natl Acad Sci U S A. 2002;99(10):6949–54.
44. Lavigne P, Najmanivich R, Lehoux JG. Mammalian StAR-related lipid transfer (START) domains with specificity for cholesterol: structural conservation and mechanism of reversible binding. Sub-cell Biochem. 2010;51:425–37.
45. Murcia M, Faraldo-Gomez JD, Maxfield FR, Roux B. Modeling the structure of the StART domains of MLN64 and StAR proteins in complex with cholesterol. J Lipid Res. 2006;47(12):2614–30.
46. Bose HS, Whittal RM, Baldwin MA, Miller WL. The active form of the steroidogenic acute regulatory protein, StAR, appears to be a molten globule. Proc Natl Acad Sci U S A. 1999;96(13):7250–5.
47. Friedland N, Liou HL, Lobel P, Stock AM. Structure of a cholesterol-binding protein deficient in Niemann-Pick type C2 disease. Proc Natl Acad Sci U S A. 2003;100(5):2512–7.
48. Im YJ, Raychaudhuri S, Prinz WA, Hurley JH. Structural mechanism for sterol sensing and transport by OSBP-related proteins. Nature. 2005;437(7055):154–8.
49. Roderick SL, Chan WW, Agate DS, Olsen LR, Vetting MW, Rajashankar KR, et al. Structure of human phosphatidylcholine transfer protein in complex with its ligand. Nat Struct Biol. 2002;9(7):507–11.
50. Kudo N, Kumagai K, Tomishige N, Yamaji T, Wakatsuki S, Nishijima M, et al. Structural basis for specific lipid recognition by CERT responsible for nonvesicular trafficking of ceramide. Proc Natl Acad Sci U S A. 2008;105(2):488–93.
51. Soccio RE, Adams RM, Romanowski MJ, Sehayek E, Burley SK, Breslow JL. The cholesterol-regulated StarD4 gene encodes a StAR-related lipid transfer protein with two closely related homologues, StarD5 and StarD6. Proc Natl Acad Sci U S A. 2002;99(10):6943–8.
52. Rodriguez-Agudo D, Calderon-Dominguez M, Ren S, Marques D, Redford K, Medina-Torres MA, et al. Subcellular localization and regulation of StarD4 protein in macrophages and fibroblasts. Biochim Biophys Acta. 2011;1811(10):597–606.

53. Soccio RE, Adams RM, Maxwell KN, Breslow JL. Differential gene regulation of StarD4 and StarD5 cholesterol transfer proteins. Activation of StarD4 by sterol regulatory element-binding protein-2 and StarD5 by endoplasmic reticulum stress. J Biol Chem. 2005;280(19):19410–8.
54. Leventis PA, Grinstein S. The distribution and function of phosphatidylserine in cellular membranes. Annu Rev Biophys. 2010;39:407–27.
55. Fridriksson EK, Shipkova PA, Sheets ED, Holowka D, Baird B, McLafferty FW. Quantitative analysis of phospholipids in functionally important membrane domains from RBL-2H3 mast cells using tandem high-resolution mass spectrometry. Biochemistry. 1999;38(25):8056–63.
56. Bose M, Whittal RM, Miller WL, Bose HS. Steroidogenic activity of StAR requires contact with mitochondrial VDAC1 and phosphate carrier protein. J Biol Chem. 2008;283(14):8837–45.
57. Yamada S, Yamaguchi T, Hosoda A, Iwawaki T, Kohno K. Regulation of human STARD4 gene expression under endoplasmic reticulum stress. Biochem Biophys Res Commun. 2006;343(4):1079–85.
58. Riegelhaupt JJ, Waase MP, Garbarino J, Cruz DE, Breslow JL. Targeted disruption of steroidogenic acute regulatory protein D4 leads to modest weight reduction and minor alterations in lipid metabolism. J Lipid Res. 2010;51(5):1134–43.
59. Rodriguez-Agudo D, Ren S, Hylemon PB, Redford K, Natarajan R, Del Castillo A, et al. Human StarD5, a cytosolic StAR-related lipid binding protein. J Lipid Res. 2005;46(8):1615–23.
60. Rodriguez-Agudo D, Ren S, Hylemon PB, Montanez R, Redford K, Natarajan R, et al. Localization of StarD5 cholesterol binding protein. J Lipid Res. 2006;47(6):1168–75.
61. Bose HS, Whittal RM, Ran Y, Bose M, Baker BY, Miller WL. StAR-like activity and molten globule behavior of StARD6, a male germ-line protein. Biochemistry. 2008;47(8):2277–88.
62. Chen YC, Meier RK, Zheng S, Khundmiri SJ, Tseng MT, Lederer ED, et al. Steroidogenic acute regulatory-related lipid transfer domain protein 5 localization and regulation in renal tubules. Am J Physiol Renal Physiol. 2009;297(2):F380–8.
63. Letourneau D, Lorin A, Lefebvre A, Cabana J, Lavigne P, Lehoux JG. Thermodynamic and solution state NMR characterization of the binding of secondary and conjugated bile acids to STARD5. Biochim Biophys Acta. 2013;1831(11):1589–99.
64. Borthwick F, Allen AM, Taylor JM, Graham A. Overexpression of STARD3 in human monocyte/macrophages induces an anti-atherogenic lipid phenotype. Clin Sci. 2010;119(7):265–72.
65. Tsai YC, Weissman AM. The unfolded protein response, degradation from endoplasmic reticulum and cancer. Genes Cancer. 2010;1(7):764–78.
66. Golas A, Malek P, Piasecka M, Styrna J. Sperm mitochondria diaphorase activity–a gene mapping study of recombinant inbred strains of mice. Int J Dev Biol. 2010;54(4):667–73.
67. Alpy F, Stoeckel ME, Dierich A, Escola JM, Wendling C, Chenard MP, et al. The steroidogenic acute regulatory protein homolog MLN64, a late endosomal cholesterol-binding protein. J Biol Chem. 2001;276(6):4261–9.
68. Miller WL. StAR search–what we know about how the steroidogenic acute regulatory protein mediates mitochondrial cholesterol import. Mol Endocrinol. 2007;21(3):589–601.
69. Rone MB, Fan J, Papadopoulos V. Cholesterol transport in steroid biosynthesis: role of protein-protein interactions and implications in disease states. Biochim Biophys Acta. 2009;1791(7):646–58.
70. Hauet T, Yao ZX, Bose HS, Wall CT, Han Z, Li W, et al. Peripheral-type benzodiazepine receptor-mediated action of steroidogenic acute regulatory protein on cholesterol entry into leydig cell mitochondria. Mol Endocrinol. 2005;19(2):540–54.
71. Tomasetto C, Regnier C, Moog-Lutz C, Mattei MG, Chenard MP, Lidereau R, et al. Identification of four novel human genes amplified and overexpressed in breast carcinoma and localized to the q11-q21.3 region of chromosome 17. Genomics. 1995;28(3):367–76.
72. Alpy F, Tomasetto C. Give lipids a START: the StAR-related lipid transfer (START) domain in mammals. J Cell Sci. 2005;118(Pt 13):2791–801.

73. Strauss JF 3rd, Kishida T, Christenson LK, Fujimoto T, Hiroi H. START domain proteins and the intracellular trafficking of cholesterol in steroidogenic cells. Mol Cell Endocrinol. 2003;202(1–2):59–65.
74. Wang ML, Motamed M, Infante RE, Abi-Mosleh L, Kwon HJ, Brown MS, et al. Identification of surface residues on Niemann-Pick C2 essential for hydrophobic handoff of cholesterol to NPC1 in lysosomes. Cell Metab. 2010;12(2):166–73.
75. Zhang M, Liu P, Dwyer NK, Christenson LK, Fujimoto T, Martinez F, et al. MLN64 mediates mobilization of lysosomal cholesterol to steroidogenic mitochondria. J Biol Chem. 2002;277(36):33300–10.
76. Kishida T, Kostetskii I, Zhang Z, Martinez F, Liu P, Walkley SU, et al. Targeted mutation of the MLN64 START domain causes only modest alterations in cellular sterol metabolism. J Biol Chem. 2004;279(18):19276–85.
77. Kanno K, Wu MK, Scapa EF, Roderick SL, Cohen DE. Structure and function of phosphatidylcholine transfer protein (PC-TP)/StarD2. Biochim Biophys Acta. 2007;1771(6):654–62.
78. Hanada K, Kumagai K, Tomishige N, Yamaji T. CERT-mediated trafficking of ceramide. Biochim Biophys Acta. 2009;1791(7):684–91.
79. Wirtz KW, Devaux PF, Bienvenue A. Phosphatidylcholine exchange protein catalyzes the net transfer of phosphatidylcholine to model membranes. Biochemistry. 1980;19(14):3395–9.
80. Johnson LW, Zilversmit DB. Catalytic properties of phospholipid exchange protein from bovine heart. Biochim Biophys Acta. 1975;375(2):165–75.
81. Wustner D, Mukherjee S, Maxfield FR, Muller P, Herrmann A. Vesicular and nonvesicular transport of phosphatidylcholine in polarized HepG2 cells. Traffic. 2001;2(4):277–96.
82. Durand S, Angeletti S, Genti-Raimondi S. GTT1/StarD7, a novel phosphatidylcholine transfer protein-like highly expressed in gestational trophoblastic tumour: cloning and characterization. Placenta. 2004;25(1):37–44.
83. Horibata Y, Sugimoto H. StarD7 mediates the intracellular trafficking of phosphatidylcholine to mitochondria. J Biol Chem. 2010;285(10):7358–65.
84. Olayioye MA, Hoffmann P, Pomorski T, Armes J, Simpson RJ, Kemp BE, et al. The phosphoprotein StarD10 is overexpressed in breast cancer and cooperates with ErbB receptors in cellular transformation. Cancer Res. 2004;64(10):3538–44.
85. Olayioye MA, Vehring S, Muller P, Herrmann A, Schiller J, Thiele C, et al. StarD10, a START domain protein overexpressed in breast cancer, functions as a phospholipid transfer protein. J Biol Chem. 2005;280(29):27436–42.
86. Hanada K, Kumagai K, Yasuda S, Miura Y, Kawano M, Fukasawa M, et al. Molecular machinery for non-vesicular trafficking of ceramide. Nature. 2003;426(6968):803–9.
87. van Meer G, Holthuis JC. Sphingolipid transport in eukaryotic cells. Biochim Biophys Acta. 2000;1486(1):145–70.