Chapter 1
An Introduction to the Steroidogenic Acute Regulatory Protein (StAR)-Related Lipid Transfer Domain Protein Family

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Abstract The steroidogenic acute regulatory protein (StAR)-related lipid transfer (START) domain superfamily comprises a diverse group of proteins that bind hydrophobic lipids. The distinguishing feature shared by all members of this family is an α/β helix-grip fold structure containing a long hydrophobic pocket for ligand binding. The mammalian START domain protein family is grouped into 6 subfamilies that bind either cholesterol and oxysterols (STARD1/D3 and STARD4 subfamilies) or phospholipids and sphingolipids (STARD2/D11 subfamily), or have putative functions in Rho-GTPase signaling (STARD8/12/13 subfamily), thioesterase activity (STARD14/15 subfamily), or kinesin motor activity (STARD9). StAR (STARD1) is the namesake of the START domain protein family and has a well-established function in cholesterol transport in the adrenal and gonads for steroid hormone biosynthesis. Some of the mammalian START family members, e.g., STARD1, STARD11, and STARD2 are well characterized for their roles in cholesterol, ceramide, and phosphatidylcholine transfer, respectively, while much remains to be learned about the remaining family members. The purpose of this book is to present a compendium of the history of the discovery and the characterization of the mammalian START proteins, encompassing the seminal work over the past 50 years that has led to our current understanding of these lipid transport proteins. The chapters in this book focus on members of the STARD1/3 and STARD4 subfamilies, which have established roles involved in cholesterol and sterol trafficking. Each chapter provides a personal perspective of the discovery-to-publication journey for work on a START domain family member by authors whose work was instrumental in their discovery and characterization. This introductory chapter provides a brief overview and background on all members of the mammalian START protein family to provide a complete picture of this family of lipid transport proteins.

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Abbreviations

ACAT	acyl-CoA:cholesterol acyl transferase
ACOT	acyl-CoA thioesterase
BFIT2	brown fat-inducible thioesterase-2
CDCA	chenodeoxycholic acid
CERT	ceramide transfer protein
COL4A3BP	collagen type IV alpha 3 binding protein
DLC	deleted in liver cancer
FFAT	peptide EFFDAxE
FHA	forkhead-associated phosphopeptide binding domain
MENTAL	MLN64-N terminal domain
MLN64	metastatic axillary lymph node 64 kDa protein
NPC	Niemann –Pick type C disease
PC	phosphatidylcholine
PCTP	phosphatidylcholine transfer protein
PH	pleckstrin homology domain
SAM	sterile alpha domain
SREBP-2	sterol regulatory element binding protein-2
StAR	steroidogenic acute regulatory protein
START	StAR-related lipid transfer domain

Introduction

The StAR-related lipid transfer domain, abbreviated START, was first described by Ponting and Aravind [1] as a region of sequence similarity shared between a rat RhoGAP protein, plant Gla2 family members, mouse and human StAR, and bovine phosphatidylcholine transfer protein. Thus, the START domain was identified as a result of the testing of Web-based resources for the predictive value in identifying putative functional domains based on primary sequence data. The START domain is approximately 210 amino acids long and a distinguishing feature of this domain is the 3-D α/β helix-grip-fold structure defined by an antiparallel β-sheet flanked by amino- and carboxyl-terminal alpha helices [2]. The α/β helix-grip fold of the START domain proteins forms a U-shaped hydrophobic cleft that binds the ligand with the carboxyl-terminal alpha helix serving as a "cap" over the ligand binding cleft. Lipid access to the hydrophobic binding pocket requires a conformational change in the START domain and movement of the C-terminal helix [3, 4]. The helix-grip fold is used to define a large superfamily of proteins that bind hydrophobic lipids, classified as the SRPBCC[1] protein superfamily on NCBI's conserved domain database [5].

[1] NCBI cl14643: SRPBCC is the START/RHO_alpha_C/PITP/Bet_v1/CoxG/CalC superfamily. Rho_alpha_C, the C-terminal catalytic domains of the alpha oxygen-

START domains have been identified in plant, bacteria, protist, and animal genomes, with protein expression confirmed only in plant and animal species. Eighty percent of the START family is within the plant genome, in proteins that contain a homeodomain, suggesting a role in transcription [6]. Indeed, it is common to find START domains as part of multi-domain proteins that provide additional functions such as protein localization, enzymatic activity, or signaling [1, 2].

The mammalian START domain protein family is divided into six subfamilies based on sequence similarities [1, 7] (Table 1.1). In total, there are 15 members of the mammalian START protein family. Members of each subfamily share either similar ligand binding specificities or functional domains other than the START domain, such as the cholesterol and oxysterol binding proteins of the STARD1/D3 and STARD4/D5/D6 subfamilies, the phospholipid and sphingolipid binding proteins of the STARD2(PCTP)/D7/D10/D11 subfamily, the multi-domain proteins containing either putative Rho-GTPase signaling function of the STARD8/12/13 subfamily, thioesterase activity of the STARD14/15 subfamily, or kinesin motor function for STARD9. The first crystal structures for the START domains were reported for two mammalian START proteins, human STARD3/MLN64 and mouse STARD4 [8, 9]. Crystal structures for the START domains of hSTARD1, mSTARD4, hSTARD5, hSTARD2/PCTP, STARD11/CERT, hSTARD13, and hSTARD14 confirm the basic 3-D helix-grip fold structure across the five mammalian subfamilies [10–13]. One-third of the mammalian START domain proteins belong to the STARD1/D3 and STARD4 subfamilies and function to bind and transport cholesterol.

A brief background on the mammalian START domain family members is provided to complete the picture on these important lipid transporters (reviewed in [14, 15]). The remaining chapters of this book provide more detail on the regulation and function of members of the STARD1/D3 and STARD4 subfamilies.

The STARD1/STARD3 Subfamily

The STARD1 subfamily has two members, StAR (STARD1) and MLN64 (STARD3). StAR is the namesake of the START domain protein family and the deduced primary amino acid sequence was submitted to the NCBI database in November 1994 [16]. Shortly thereafter, a newly cloned and uncharacterized transcript from breast cancer, MLN64, was identified using differential screening of a complementary deoxyribonucleic acid (cDNA) library for amplified products in breast cancer-derived metastatic axillary lymph nodes (MLN). Protein database searches identified a domain within MLN64 that shared 33% sequence identity and 53% sequence similarity with the human StAR START domain [17, 18]. The significance

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ase subunit of Rieske-type non-heme iron aromatic ring-hydroxylating oxygenases; PIPT, phosphatidylinositol transfer proteins; Bet v 1, the major pollen allergen of white birch, Betula verrucosa; CoxG, carbon monoxide dehydrogenase subunit G (gram-negative bacteria); CalC, and related proteins.

Table 1.1 Summary of the mammalian START domain family. The START protein subfamily is indicated and each member is designated by protein name followed by other known common name(s). A schematic of the domain structure highlights that the START domain is the C-terminal domain. Tissue distribution, cellular localization, lipid binding, and functional pathways and disease association are based on data from the cited references: Tissue distribution. [a]ubiquitous expression with the major tissues studied listed; [b]restricted expression [7, 19, 26, 28, 31, 33, 49, 50, 56, 59, 60, 69] cellular location; [c]motifs, domains direct subcellular location; [d]based on immunohistochemistry data for endogenous protein expression; [e]based on *in vitro* activity; [f]based on structure; lipid binding; [g]direct ligand binding assay; [h]modeled based on structure; [i]based on *in vitro* lipid extraction assay; [j]shown in crystal [9, 11–13, 29–31, 39, 43, 70–73]; function and disease association, [1][19]; [2][22]; [3][28]; [4][26, 74, 75]; [5][36]; [6][47]; [7–9][56]; [10,11][59] [12][42], [13][66]

START Subfamily	START protein	Domain structure	Tissue distribution[a,†]	Cellular location	Lipid binding	Metabolic pathway	Disease association
STARD1/D3	STARD1 (StAR)	SS START	Adrenal, ovary, testis, brain[†], heart, liver	Mitochondria[‡,a,b,c]	Cholesterol[d,e]	steroidogenesis[1]	Lipoid CAH, HCC
	STARD3 (MLN64)	MENTAL-START	Placenta, breast, macrophages*	Transmembrane, late endosomes[‡,a,b,c]	Cholesterol[d,e]	endosomal cholesterol efflux [2]	NPC
STARD4	STARD4	START	Liver, macrophages, kidney[†]	Cytosolic[a] >> ER[a,b] mitochondria[b]	Cholesterol[¥,d]	ACAT activation[3]	
	STARD5	START	Macrophage, kidney proximal tubules[†]	Cytosolic >>ER, Golgi,PM [a]	CDCA[¥,d] Cholesterol, 25HC[¥,d]	ER stress response [4] ?	diabetic nephropathy
	STARD6	START	Testis germ cell [†]	Cytosolic[c], mitochondria[b]	Cholesterol[d,e]	?	
STARD2	STARD2 (PC-TP)	START	Liver, lung*	ER/Golgi[‡,b]	PC[f]	Glycolysis [5], FA synthesis [5]	insulin resistance
	STARD7 (GTT-1) STARD7-I	START	Liver*	Cytosolic[c] mitochondria[c]	PC[e]	?	ovarian, lung, colon, liver cancer
	STARD10	START	Liver, kidney, testis, colon*	Cytosolic[c]	PC > PE[e]	bile acid conjugation/secretion[12]	fatty liver [12]
	STARD11 (CERT)	PH FFAT START	Liver*	ER/Golgi[‡,b]	Ceramide[¥,e]	ER -> Golgi ceramide transport[6]	
STARD8/12/13	STARD8 (DCL-3)	RhoGAP START	Cancer*	Focal adhesions[‡]	?	Tumor suppressor[7]	
	STARD12 (DCL-1)	SAM RhoGAP START	Cancer*	Focal adhesions[‡] PM[a]	?	Cytoskeletal organization[8] Tumor suppressor8	liver cancer
	STARD13 (DCL-2)	SAM RhoGAP START	Endothelial cells*	Focal adhesions[‡]	Charged lipid[d] (?)	Tumor suppressor[9]	angiogenesis
STARD9	STARD9	KM FHA CC START	ubiquitous	nuclear	?	mitotic spindle formation [13]	mitosis
STARD14/15	STARD14 (ACOT11_v2, BFIT2)	Hotdog Hotdog START	Brown adipose tissue[†]	Cytosolic[c], ?	Fatty acid[d] (?)	Medium chain fatty-acyl-coA hydrolysis [10]	thermogenesis
	STARD15 (ACOT12)	Hotdog Hotdog START	Liver[‡]	Cytosolic[c]	?	Acetyl-coA hydrolysis [11]	

of the START domain in MLN64 suggested a function in cholesterol binding and/or trafficking. Overall, the two members of the STARD1/D3 subfamily are similar in that the START domain for both proteins binds only cholesterol and additional sequences or domains localize the proteins to specific subcellular compartments. The differential subcellular localization of these START proteins suggests different functions in cholesterol trafficking (Table 1.1). First, StAR regulates cholesterol transfer into mitochondria and controls steroid hormone biosynthesis in the adrenal and gonads (reviewed in [19]). It is a nuclear-encoded protein that is synthesized in the cytosol as a 37 kDa precursor protein with an N-terminal mitochondria targeting sequence that directs the protein to the mitochondria. Mitochondrial import and processing of the precursor produces a 32 kDa intermediate product and a mature 30 kDa form that is localized within the matrix. The history and regulation of StAR in steroidogenic tissues is the topic for Chap. 2 while START domain structure and current models for cholesterol transport by StAR are the topics for Chaps. 3 and 4, respectively. Early studies indicated that StAR could transport cholesterol across mitochondrial membranes in many cell types, suggesting that StAR may function outside of steroidogenic tissues [20]. The action of StAR in non-steroidogenic cells is the topic for Chap. 5.

STARD3/MLN64 is a transmembrane protein that is targeted to the late endosomes. The location of STARD3/MLN64 to late endosomes led to studies on its potential role in Niemann Pick type C (NPC) disease, a lipid storage disorder caused by mutations in genes encoding either NPC1 or NPC2 that result in accumulation of cholesterol in lysosomes (reviewed in [21]). STARD3/MLN64 may function to shuttle cholesterol from NPC1 to a cytosolic acceptor protein or to an adjacent membrane [22–25]. STARD3 expression and function is the topic for Chap. 6.

The STARD4 Subfamily

STARD4 was first described as part of a gene set that was downregulated in mouse liver as a consequence of a high cholesterol diet. Five of the six genes that were identified with >2-fold decreased expression after high cholesterol diet were established cholesterol-regulated genes, and one was an uncharacterized EST (expressed sequence tag; [7]). Subsequent sequence and cloning studies confirmed that the EST was part of a transcript that contained a START domain; in fact the deduced amino acid sequence of the open reading frame encoded a protein of 224 amino acids composed entirely of the START domain. This EST was named STARD4.

Fifteen START domain-containing genes were identified in the human genome, 12 of which were previously characterized and two that were uncharacterized yet had 26–32 % sequence identity with STARD4. Based on the high sequence identity with STARD4, these genes were named sequentially STARD5 and STARD6 and formed the three members of the STARD4 subfamily [7]. The STARD4 family members represent ~22 kDa soluble proteins with no membrane targeting/association predicted (Table 1.1). STARD4 and STARD5 transcripts are abundant in liver

and kidney tissues. In the liver, STARD4 is found in the hepatocytes and Kupffer cells, the macrophages of the liver, while STARD5 is detected only in Kupffer cells. In kidney, both STARD4 and STARD5 protein are present in the proximal tubules [26].

STARD4 binds only cholesterol with a proposed biological role for STARD4 in regulating cholesterol sensing by the endoplasmic reticulum and cholesterol ester synthesis [27–29]. STARD5 binds primary bile acids, specifically chenodeoxycholic acid, with high affinity [30]. The fact that STARD5 is a bile acid binding/transporting START protein was a surprise to the field given that initial reports suggested cholesterol and 25-hydroxycholesterol were the ligands for STARD5 [7, 27, 29, 31]. The potential biological functions for StARD5 appear to be different from those of STARD4, in that STARD5 expression is not linked to increased cholesterol ester levels but rather to increased free cholesterol levels [26, 29, 31, 32]. It remains to be determined whether STARD5 plays a role in cholesterol homeostasis, yet it is clear that both direct and indirect mechanisms should be explored. For example, STARD5 may modulate the activity of the bile acid-activated nuclear receptor farnesol-X-receptor (FXR) by either promoting or blocking ligand binding. FXR controls expression of genes involved in cholesterol, bile acid, and lipid homeostasis, thus, STARD5 could potentially indirectly regulate FXR-dependent signaling. Chapters 7 and 8 present the seminal work on STARD4 and STARD5 expression, regulation, and function, and explores further the current questions on the role of STARD5 in cholesterol homeostasis.

STARD6 is expressed in mouse testis, specifically in the germ cells with highest expression in round spermatids [7, 33]. The function of STARD6 in spermatogenesis is not known, although it may play a role in mitochondrial NADH-dependent dehydrogenase activity (diaphorase) associated with sperm motility and quality [34].

The STARD2/Phosphatidylcholine Transfer Protein (PCTP) Subfamily

STARD2, STARD7, and STARD10 all bind phosphatidylcholine (PC) with STARD2 being the best characterized PC transporter [12]. The early work on STARD2, also named phosphatidylcholine transfer protein (PCTP), tested its effect on PC transport in the liver and lung, reasoning that the protein is highly expressed in these tissues where PC is a major phospholipid in bile and lung surfactant [35]. The direct test was to generate mice deficient in STARD2/PCTP (*Pctp*−/−) and measure PC in the bile and lung surfactant [35]. The unpredicted result was the *Pctp*−/− mice have the same bile or lung surfactant PC content as their wild-type counterparts. However, the investigators continued to characterize these mice and made an interesting observation that fasting serum glucose and free fatty acids levels were significantly decreased in *Pctp*−/− mice [36]. Similarly, inhibiting PC binding to STARD2/PCTP in wild-type mice decreased the effects of a high fat diet on serum glucose levels [37]. Blocking STARD2/PCTP action, i.e., PC binding in cultured human

hepatocytes activated the insulin signaling pathway [37]. Therefore, it appears that STARD2/PCTP functions in the liver to suppress insulin sensitivity. This unpredicted role for STARD2 opens new possibilities for targeting STARD2 in treatment of diabetes. Future studies are necessary to determine the mechanism for STARD2/PCTP action in liver glucose metabolism.

STARD7 also binds PC specifically and shares 25% sequence identity with STARD2/PCTP [38, 39]. The protein has been detected in lung, colon, and liver cancer cell lines [38], and therefore, has tissue overlap with STARD2. A STARD7 variant, STARD7-1, with an amino terminal mitochondrial targeting sequence has been associated with increased mitochondrial PC levels when overexpressed in a cultured mouse hepatoma cell line [39]. Both cytosolic and mitochondrial localization of endogenous STARD7-I in liver cells and tissue has been demonstrated, consistent with the increased PC levels in mitochondria with overexpression of the protein. Currently, it is proposed that STARD7 may play a role in mitochondrial biogenesis, yet this has not been directly addressed [39].

STARD10 expression has been reported in mouse testicular germ cells, liver, intestine, and human mammary tissue [40–42]. PC and phosphatidylethanolamine both bind STARD10 although PC binds with greater affinity [40, 43]. The PC transfer activity of STARD10 appears to be regulated by posttranslational modification; phosphorylation of STARD10 decreases membrane association and PC extraction [44]. A putative role in breast cancer has been proposed with STARD10 functioning within the ErbB2/HER2/neu receptor signaling pathway, although the coordinated expression of STARD10 and HER2/neu in breast tumors may not be directly associated [40, 45]. Additionally, loss of STARD10 expression was found to be an independent marker for poor patient outcome and may be used to identify a specific subgroup of patients at high risk [45]. Presently, the function of STARD10 in mammary tissues is not defined so the question remains whether it contributes to the anti- or pro-oncogenic breast tumor phenotype. The newly described *Stard10* knockout mice (Stard10$^{-/-}$) may serve as a good model to address this question directly [42]. Interestingly, characterization of the Stard10$^{-/-}$ mice provided some unexpected results; the levels of PC in the liver and bile were not different between the wild-type and the Stard10$^{-/-}$ mice, rather the major disorder was in bile acid metabolism with an increase in biliary secretion of conjugated bile acids. Much of the change in bile acid metabolism is attributed to the peroxisome proliferator-activated receptor-alpha (PPARα)-mediated changes in gene expression in the liver and intestine [42].

STARD11 is more commonly referred to as CERT, for ceramide transport protein. STARD11/CERT is responsible for the movement of ceramide from the ER to the Golgi membrane [46]. CERT is a multi-domain protein containing an amino terminal pleckstrin homology domain (PH), a FFAT motif, and carboxyl-terminal START domain. The domain structure of CERT orients the protein between ER and Golgi membranes; the PH domain binds to the phosphoinositide PI4P in the Golgi membrane and the FFAT motif binds to a vesicle-associated membrane protein-associated protein (VAP), an integral ER membrane protein. This orientation of the protein models the START domain in close proximity to both membranes so that

association with the ER extracts ceramide from the membrane and association with the Golgi promotes ceramide absorption by the membrane [10, 11, 47]. Within this subfamily, STARD2/PCTP and STARD11/CERT have been studied in greater detail and there are several reviews on their potential functions [47–50].

The STARD8/12/13 Subfamily

The members of this subfamily contain a sterile alpha motif [13], a serine-rich region, a RhoGAP domain, and a START domain that make this subfamily the most complex in terms of domain structure ([13] Table 1.1). STARD12, the first member identified within this family, is contained in a genomic region that is associated with loss of heterozygosity in several cancers, hence, members of this subfamily are also referred to as the *d*eleted in *l*iver *c*ancer (DLC) family of proteins [51]. STARD12/DLC-1, STARD13/DLC-2 and STARD8/DLC-3 all function as tumor suppressors based on studies that showed increased apoptosis and decreased cell growth in several cancer cell lines after re-expression of one of the proteins [52, 53]. The basis for the tumor suppressor function is not known, yet the multi-domain nature of these proteins and the subcellular localization provide clues to function and suggest a potential for integrated functions. For example, imaging studies in live cells have shown that the STARD12/13/8 proteins are present at the plasma membrane, at regions associated with cytoskeletal proteins (focal adhesions) and lipid rafts enriched in cholesterol and sphingolipid [54, 55]. The lipid that binds the START domain in this family has not been determined, although the crystal structure for STARD13/DLC-2 predicts the ligand binding pocket would accommodate a charged lipid rather than cholesterol or a phospholipid [13]. The location of these proteins at the plasma membrane leads to speculation that ligand binding in the START domain may regulate the RhoGAP function, thereby controlling Rho signaling and cell proliferation [55, 56].

The STARD14/15 Subfamily

STARD14 and STARD15 contain an ACOT (acyl-coenzyme A thioesterase) domain in addition to the START domain and functions in the hydrolysis of coenzyme A [57] from activated fatty acids and acetyl-CoA [58, 59]. Human STARD14 is the ortholog of the mouse brown fat-inducible thioesterase (mBFIT2; [60] Table 1.1) and its expression is associated with increased metabolic activity. STARD15/ACOT12 is a cytosolic acetyl-CoA thioesterase (hydrolase) that is highly expressed in liver [61–63]. The crystal structure for the START domain of STARD14 predicts [13] a fatty acid would fill the ligand-binding cavity. Although neither the ligand nor role of the START domain is known for this subfamily, the evolution of the two domain structure would indicate a convergence of purpose. One model is that fatty

acid binding in the START domain regulates the thioesterase activity, similar to that proposed for the START domain-RhoGAP interaction of the STARD8/12/13 subfamily.

The STARD9 Subfamily

The STARD9 subfamily contains a single member which has an amino-to-carboxy terminal multi-domain structure composed of a kinesin motor domain, a forkhead-associated phosphopeptide binding domain (FHA) and the START domain [7, 64, 65]. The STARD9 kinesin and FHA domains have sequence similarity (~50% identity) to the kinesin-3 family members KIF16B and KIF1A. STARD9 was identified as a protein that associated with mitotic microtubules and the amino terminal kinesin domain of STARD9 is active for microtubule binding and adenosine triphosphate (ATP) hydrolysis, supporting STARD9 functions as a kinesin motor protein. Small interfering ribonucleic acid (siRNA) depletion of STARD9 in several cancer cell lines revealed the protein is important for microtubule spindle assembly and mitosis; loss of STARD9 resulted in increased mitotic arrest and increased apoptosis [66]. The loss of STARD9 also enhanced the sensitivity of the cells to anti-mitotic cancer drugs, implicating STARD9 as a putative novel target for cancer treatment. However, both the identity of the lipid bound by the START domain and the function of lipid binding for STARD9 function remains to be determined.

Summary

The START domain family is composed of lipid transport proteins that function in the non-vesicular trafficking of sterols and phospho/sphingolipids. The STARD1/D3 subfamily members are established cholesterol binding/transport proteins, yet defining the mechanisms of sterol transport for these proteins remains a current challenge. StAR, the START protein family namesake, regulates cholesterol transport across mitochondrial membranes for steroid hormone synthesis and defining the mechanism(s) for StAR-dependent cholesterol transport has been investigated using both structural and functional approaches. Current models for mitochondrial cholesterol transport by StAR are presented in Chaps. 3 and 4 while the potential for an expanded role for StAR in non-steroidogenic tissues is presented in Chap. 5. STARD3 is a membrane protein localized to the late endosome and has a putative role in shuttling cholesterol derived from exogenous sources to cytosolic cholesterol carriers for subsequent cellular distribution. Chapter 6 provides the discovery-to-current models for function journey for STARD3.

The tissue distribution profiles, gene regulation, and ligand specificities suggest unique functions for the STARD4 family members. As soluble lipid transport proteins, this subfamily is proposed to participate in the non-vesicular trafficking of

cholesterol between biological membranes to help maintain the proper cholesterol: phospholipid:sphingolipid distribution (reviewed in [67, 68]). Most of the work reported has been on STARD4 and the regulation and function of STARD4 is the topic for Chaps. 7 and 8 while a general overview of non-vesicular cholesterol trafficking and the role of the STARD4 subfamily in this process is the topic for Chap. 8.

Functions described for members of the remaining START subfamilies reveal a very diverse role for this family of proteins. The knockout mouse models for members of the STARD2 phospholipid/sphingolipid binding proteins have provided unexpected and interesting results that indicate potential broad applications for this subfamily in regulating glucose metabolism in diabetes (STARD2), tumor proliferation in cancer (STARD7/10), bile acid metabolism in the liver and intestine (STARD10), and ceramide transfer from the ER to the Golgi membrane for sphingolipid synthesis (STARD11/CERT). Although PC phospholipid metabolism or trafficking appears to be normal in the absence of STARD2 or STARD10, a remaining question is whether lipid binding to the START domain impacts the function of these proteins. The lipid specificity for the remaining START proteins in the RhoGAP multi-domain subfamily, the thioesterase subfamily, and the STARD9 subfamily has not been determined. The RhoGAP multidomain START proteins are defined by their loss in cancer and can function as tumor suppressors although this function is attributed to the RhoGAP domain. The thioesterase START protein subfamily functions in fatty acid hydrolysis while STARD9 binds to centromeres and is important for mitotic spindle formation, again both functions attributed to other domains within the proteins. It is plausible to predict that lipid binding in the START domains will regulate the described function for these proteins, and testing this prediction will require identifying the lipid ligands.

Lastly, as disease states can provide significant insight into biological function, START protein expression and function should be considered when examining disorders that involve dyslipidemia and inflammation. Since dyslipidemia and inflammation are common to obesity, diabetes, coronary heart disease, and cancer, there is much to be learned about this family of lipid transport proteins.

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