The mammalian START domain protein family in lipid transport in health and disease

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Abstract

Lipid transfer proteins of the steroidogenic acute regulatory protein-related lipid transfer (START) domain family are defined by the presence of a conserved ~210 amino acid sequence that folds into an \( \alpha/\beta \) helix-grip structure forming a hydrophobic pocket for ligand binding. The mammalian START proteins bind diverse ligands, such as cholesterol, oxysterols, phospholipids, sphingolipids, and possibly fatty acids, and have putative roles in non-vesicular lipid transport, thioesterase enzymatic activity, and tumor suppression. However, the biological functions of many members of the START domain protein family are not well established. Recent research has focused on characterizing the cell-type distribution and regulation of the START proteins, examining the specificity and directionality of lipid transport, and identifying disease states associated with dysregulation of START protein expression. This review summarizes the current concepts of the proposed physiological and pathological roles for the mammalian START domain proteins in cholesterol and lipid trafficking.

Introduction

Lipid transport proteins play an important role in non-vesicular trafficking of cholesterol, phospholipids, and sphingolipids between biological membranes to help maintain the proper cholesterol:phospholipid:sphingolipid distribution (reviewed in Prinz (2007) and Lev (2010)). Cholesterol content is maintained at relatively low levels within the endoplasmic reticulum (ER) and mitochondrial membranes compared with the plasma membrane (PM; Mesmin & Maxfield 2009). The source of PM cholesterol is from both de novo synthesis in the ER and cellular uptake of low-density lipoprotein (LDL)-derived cholesterol. De novo-synthesized cholesterol is rapidly transferred to the PM by non-vesicular trafficking mechanism(s), implicating a role for soluble sterol transport proteins (Maxfield & van Meer 2009). Receptor-mediated endocytosis of LDL delivers lipoproteins to the late endosomes/lysosomes where free cholesterol is hydrolyzed from cholesterol esters (Goldstein & Brown 2009). The free cholesterol is recycled back to the PM or transported to the ER. In the PM, cholesterol can be found clustered with sphingolipids into detergent-resistant protein–lipid microdomains referred to as lipid rafts (Danielsen & Hansen 2003, Rajendran & Simons 2005, Hanzal-Bayer & Hancock 2007). Functionally, lipid rafts are proposed to provide an organized membrane region for signaling and other functions (Lingwood & Simons 2010). Changes in ER membrane cholesterol levels signal for changes in gene expression leading to altered cholesterol metabolism while transport of cholesterol to mitochondria is required for production of steroid hormones and bile acids. Thus, it has long been appreciated that maintaining proper cholesterol distribution within the cell is important for cholesterol homeostasis and membrane function (Qin et al. 2006, Maxfield & van Meer 2010). There are two major gene families for lipid transfer proteins with specificity for sterols: the steroidogenic acute regulatory protein (StAR)-related lipid-transfer (START) domain family and the oxysterol-binding protein (OSBP) family, which includes the OSBP-related proteins (ORPs). This review focuses on the role of the mammalian START domain family in lipid trafficking and the implications for dysregulation of START protein expression in disease states. The OSBP/ORP family has been reviewed by others (Prinz 2007, Ngo et al. 2010).

The START domain protein family

The START domain is defined by a conserved sequence of ~210 amino acids that folds into an \( \alpha/\beta \) helix-grip structure forming a hydrophobic pocket for ligand binding.
forming a hydrophobic pocket for binding sterols and other lipids (Ponting & Aravind 1999, Iyer et al. 2001). The helix-grip fold is used to define a large superfamily of START domains in genomes from plants, bacteria, protists, and animals, but not in archaea or yeast (Schrick et al. 2004). START domains are relatively rare in bacteria and protist genomes, and to date, there is no evidence that these proteins are expressed. Proteins containing the START domain are most abundant in plants and are highly represented in proteins that contain a homeodomain, suggesting a role in transcription (Schrick et al. 2004). The homeodomain–START domain structure has only been found in plant proteins. Coupling the START domain with other motifs is, however, a common theme as START domains in other phyla are found in multi-domain proteins that provide additional functions such as protein localization, enzymatic activity, or signaling (Ponting & Aravind 1999, Iyer et al. 2001).

The mammalian START domain protein family is well characterized and is composed of 15 members that group into six subfamilies based on the sequence and ligand similarities (Ponting & Aravind 1999, Soccio et al. 2002; Table 1). In very general terms, the subfamilies can be classified into three major domains: cholesterol- and oxysterol binding proteins (STARD1/D3 and STARD4/D5/D6 subfamilies), the phospholipid- and cholesterol- and oxysterol binding proteins (STARD2 (phosphatidycholine transfer protein, PCTP)/D7/D10/D11 subfamily), the multi-domain proteins containing either putative Rhodopsin-GTPase signaling function (STARD8/12/13 subfamily) or thioesterase activity (STARD14/15 subfamily), and the STARD9 subfamily composed of a single member of unknown function that is not further discussed (Soccio et al. 2002, Soccio & Breslow 2003, Alpy & Tomasetto 2005).

The crystal structures for the START domains of hSTARD3/mSTARD4 were the first to be solved and showed an α/β helix-grip fold with a nine-stranded anti-parallel β-sheet forming a U-shaped hydrophobic cleft that binds the ligand and is flanked by amino- and carboxyl-terminal α helices (Tsujishita & Hurley 2000, Romanowski et al. 2002). The carboxyl-terminal α helix is proposed to serve as a ‘cap’ to the ligand-binding site, with lipid access to the binding pocket requiring a conformational change in the START domain and movement of the C-terminal helix (Baker et al. 2005, Bose et al. 2008a,b). To date, crystal structures for the START domains of hSTARD1, hSTARD5, hSTARD2/PCTP, STARD11/CERT, hSTARD13, and hSTARD14 have been reported and the data confirm the basic three-dimensional helix-grip fold structure across the five mammalian subfamilies that defines this family of proteins (Roderick et al. 2002, Kudo et al. 2008, 2010, Thorsell et al. 2011). Modeling of START domain conformational changes and mechanisms for cholesterol absorption/desorption have been reviewed elsewhere (Alpy & Tomasetto 2005, Miller 2007, Lavigne et al. 2010).

**Cholesterol trafficking and homeostasis**

Intracellular cholesterol levels are tightly regulated by controlling biosynthetic and degradation pathways. There are several excellent reviews on cholesterol homeostasis and cholesterol trafficking (Russell 2003, Soccio & Breslow 2004, Prinz 2007, Brown & Goldstein 2009, Mesmin & Maxfield 2009, Maxfield & van Meer 2010, Wollam & Antebi 2011), and only a brief overview of these topics is provided to set the cellular context for START protein function(s). The major regulatory step for cholesterol biosynthesis is the expression and activation of the enzyme HMG-CoA reductase (HMGR). HMGR transcription is controlled by sterol regulatory element-binding protein-2 (SREBP2), a member of the basic helix–loop–helix–leucine zipper (bHLH-Zip) transcription factor family that is encoded by the SREBP2 gene (Hua et al. 1993, Yokoyama et al. 1993, Sakai & Rawson 2001). SREBP2, however, is a proteolytic fragment of a larger transmembrane protein of the ER that forms a protein complex composed of SREBP, SREBP-cleavage-activating protein (SCAP), and insulin-induced genes 1 and 2 (INSIGs) that functions as a cellular cholesterol sensor. When ER cholesterol levels decline below some threshold level, SREBP-SCAP dissociates from INSIGs and moves to the Golgi apparatus where two proteolytic cleavages releases a ~50 kDa N-terminal fragment that translocates to the nucleus and activates target gene transcription (Anderson 2003; Fig. 1). Major target genes induced by SREBP2 in the liver are within the cholesterol biosynthetic pathway including HMGR and the LDL receptor (LDLR) (Horton et al. 2003). A resulting increase in cholesterol synthesis (HMGR) and uptake (LDLR) increases intracellular cholesterol levels. The resulting increase in ER cholesterol stabilizes the INSIG-SCAP-SREBP2 complex within the ER and thereby suppresses SREBP2 processing and subsequent transcriptional function(s). Cholesterol is also converted to cholesterol esters by acyl-CoA:cholesterol acyl transferase activity (ACAT), an enzyme localized to the ER. An increase in ER cholesterol levels activates ACAT leading to increased cholesterol ester synthesis.

Cholesterol metabolism in bile acids in the liver represents the major route for cholesterol clearance. There are two pathways for bile acid biosynthesis, the classical pathway that is initiated in the cytosol and the alternative pathway in mitochondria (reviewed in Russell 2003). In liver mitochondria, cholesterol is hydroxylated on the side chain at position C27 or C25 by the cytochrome P450 enzyme CYP27A1 to produce the oxysterols 27-hydroxycholesterol (27HC) or 25-hydroxycholesterol (25HC) (Li et al. 2007). In addition to simply serving as intermediates in bile acid biosynthetic pathway, 27HC and 25HC are cellular signals.
**Table 1** Characteristics of the mammalian START domain protein family members

<table>
<thead>
<tr>
<th>START subfamily</th>
<th>START protein</th>
<th>Other name(s)</th>
<th>Domain structure</th>
<th>Tissue distribution*†</th>
<th>Cellular location</th>
<th>Lipid binding</th>
<th>Function/metabolic pathway</th>
</tr>
</thead>
<tbody>
<tr>
<td>STARD1/D3</td>
<td>STARD1</td>
<td>STAR</td>
<td>—<strong>START</strong></td>
<td>Adrenal, ovary, testis, brain†</td>
<td>Mitochondria</td>
<td>Cholesterol⁴ e</td>
<td>Steroidogenesis¹</td>
</tr>
<tr>
<td></td>
<td>STARD3</td>
<td>MLN64</td>
<td>MENTAL-START</td>
<td>Placenta, breast, macrophages*</td>
<td>Transmembrane, late endosomes</td>
<td>Cholesterol⁴ e</td>
<td>endosomal cholesterol efflux² ACAT activation³</td>
</tr>
<tr>
<td></td>
<td>STARD4</td>
<td>START</td>
<td>START</td>
<td>Liver, macrophages, kidney†</td>
<td>Cytosolic⁶ ⇒ ER⁴, mitochondria⁶</td>
<td>Cholesterol⁴ d</td>
<td>ER stress response⁴</td>
</tr>
<tr>
<td></td>
<td>STARD5</td>
<td>START</td>
<td>START</td>
<td>Macrophage kidney proximal tubules⁵</td>
<td>Cytosolic⁶ ⇒ ER, Golgi, PM⁶</td>
<td>Cholesterol, 25HC⁴, d</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>STARD6</td>
<td>START</td>
<td>—</td>
<td>Testis germ cell⁵</td>
<td>Cytosolic⁶, mitochondria⁶</td>
<td>Cholesterol⁴ e</td>
<td>—</td>
</tr>
<tr>
<td>STARD2</td>
<td>STARD2</td>
<td>PC-TP</td>
<td>START</td>
<td>Liver, lung*</td>
<td>ER/Golgi⁵, b</td>
<td>PC f</td>
<td>Glycolysis⁵, FA synthesis⁵</td>
</tr>
<tr>
<td></td>
<td>STARD7</td>
<td>GTT-1</td>
<td>—</td>
<td>Liver*</td>
<td>Cytosolic⁶</td>
<td>PC e</td>
<td>?</td>
</tr>
<tr>
<td>STARD10</td>
<td>CERT, GPBPα26, COl4A3BP</td>
<td>——START— —FFAT— START</td>
<td>Liver, kidney, testis, colon*</td>
<td>—</td>
<td>Cancer*</td>
<td>—</td>
<td>Tumor suppressor⁷</td>
</tr>
<tr>
<td>STARD11</td>
<td>RhoGAP—START</td>
<td>SAM—RhoGAP—START</td>
<td>—</td>
<td>Cancer*</td>
<td>ER/Golgi⁵, b</td>
<td>Ceramide⁶ e</td>
<td>ER → Golgi ceramide transport⁶</td>
</tr>
<tr>
<td>The RhoGAP multidomain proteins</td>
<td>STARD8</td>
<td>DLC-3</td>
<td>RhoGAP—START</td>
<td>Cancer*</td>
<td>Focal adhesions⁶</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>DLEC-1</td>
<td>RhoGAP—START</td>
<td>SAM—RhoGAP—START</td>
<td>Cancer*</td>
<td>Focal adhesions⁶ PM⁶</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>STARD9</td>
<td>STARD13</td>
<td>DLEC-2</td>
<td>SAM—RhoGAP—START</td>
<td>Endothelial cells*</td>
<td>—</td>
<td>Charged lipid⁶ (?)</td>
<td>—</td>
</tr>
<tr>
<td>STARD9</td>
<td>STARD14</td>
<td>DLEC-1</td>
<td>RhoGAP—START</td>
<td>Brown adipose tissue†</td>
<td>—</td>
<td>Cytosolic⁶ ?</td>
<td>—</td>
</tr>
<tr>
<td>The thioesterase multidomain proteins</td>
<td>STARD15</td>
<td>ACOT11_v2, BFIT2</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>STARD15</td>
<td>ACOT12</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

StAR, steroidogenic acute regulatory protein; START, STAR-related lipid transfer domain; MLN64, metastatic axillary lymph node 64 kDa protein; PC-TP, phosphatidylcholine transfer protein; GTT1, gestational trophoblastic tumor gene-1; CERT, ceramide transfer protein; GPBP, Goodpasture antigen-binding protein; COL4A3BP, collagen-type IV α3 binding protein; DLEC, deleted in liver cancer; ACOT, acyl-CoA thioesterase; BFIT2, brown fat-inducible thioesterase-2; ss, signal sequence; MENTAL, MLN64-N terminal domain; PH, pleckstrin homology domain; FFAT, peptide EFFDAE; SAM, sterile domain; Hotdog, conserved domain structure for acyl-coenzyme A thioesterase family that has thioesterase activity; ACAT, acyl-CoA:cholesterol acyl transferase activity. Cellular location: ‡domains direct subcellular location; a based on immunohistochemistry data for endogenous protein expression; b based on in vitro activity; c based on structure. Lipid binding: ddirect ligand binding assay; e modeled based on structure; f based on in vitro lipid extraction assay; g shown in crystal (Tsujishita & Hurley 2000, Roderick et al. 2002, Olayioye et al. 2005, Rodriguez-Agudo et al. 2005, Murcia et al. 2006, Bose et al. 2008a, b, Kanno et al. 2007a, b, Chen et al. 2009, Kirkby et al. 2010). Lipid binding: h restricted expression (Adams et al. 2001, Stocco 2001, Socci et al. 2002, Strauss et al. 2003, Gomes et al. 2005, Rodriguez-Agudo et al. 2005, Durkin et al. 2007a, b, Kanno et al. 2007a, b, Chen et al. 2009, Kirkby et al. 2010, Mencarelli et al. 2010, Rodriguez-Agudo et al. 2011). Note that STARD4 and STARD5 mRNA have been detected at low levels in heart; therefore, they may have a broader expression. **TABLE continued on next page.**
that help control cholesterol homeostasis by repressing cholesterol synthesis and enhancing cholesterol efflux. 27HC and 25HC bind to INSIGs and block SREBP-SCAP translocation to the Golgi, thereby repressing SREBP2-dependent pathways (cholesterol synthesis; Radhakrishnan et al. 2007, Sun et al. 2007). In addition, 27HC and 25HC are ligands for the liver X receptor alpha (LXRα), a nuclear receptor that regulates lipid metabolism to help maintain cholesterol homeostasis. LXRα directly activates transcription of the gene encoding the ATP-binding cassette A1 (ABCA1), a cholesterol transporter located in the PM that is important for cholesterol efflux from extra-hepatic cells. Another LXRα target gene is SREBP1c, another ER membrane-bound SREBP protein that has an N-terminal bHLH-Zip transcription factor that activates genes that encode enzymes in the fatty acid biosynthesis pathway.

Macrophages also convert cholesterol to 27HC in mitochondria using CYP27A1. In extra-hepatic cells, the C27 hydroxyl group of 27HC can be oxidized to a carboxylic acid generating 3β-hydroxy-5-cholestenoic acid, a soluble bile acid precursor that can be secreted and taken up by the liver where it can be further metabolized to bile acids (Babiker & Diczfalusy 1998, Bjorkhem et al. 1999). Thus, the action of CYP27A1 and production of 27HC in macrophages can decrease cellular cholesterol levels by two mechanisms; activation of the LXR-dependent pathway leading to enhanced cholesterol efflux via the PM.
ABCA1 and production of soluble 3β-hydroxy-5-cholestenolic acid. These are important adaptive responses since macrophages scavenge oxidized-LDL and accumulate lipids in the process of foam cell development. The mechanisms that modulate oxysterol production can have an impact on overall cholesterol homeostasis in liver and macrophages.

Disorders in cholesterol homeostasis are recognized as important contributors to disease states associated with dyslipidemia, e.g. atherosclerosis, fatty liver disease, diabetes, and cancer. This review highlights the current literature that implicates dysregulation of START protein expression in several of these disease states.

The cholesterol/oxysterol-binding START proteins

**STARD1/STARD3 subfamily: the membrane-targeted START proteins**

StAR is the founding member of the START domain protein family and is expressed predominantly in the adrenal and gonads where it functions to bind cholesterol and facilitate its transfer from the outer to the inner mitochondrial membrane to initiate steroid hormone biosynthesis (Clark et al. 1994, Stocco & Clark 1996). StAR is unique among START family members in that it contains a mitochondria-targeting sequence, a classical amino-terminal amphipathic helix that directs the protein to the mitochondria. It is a nuclear-encoded phosphoprotein that is synthesized in the cytosol as a 37 kDa precursor protein. Mitochondrial import and processing of the precursor produce a 32 kDa intermediate product and a mature 30 kDa form that is localized within the matrix (reviewed in Stocco 2001). Phosphorylation of the 37 kDa StAR protein at Ser194/195 (mouse/human) by protein kinase A is required for maximal activity (Arakane et al. 1997, Fleury et al. 2004, Jo et al. 2005, Dyson et al. 2008). Although the detailed mechanism for StAR-mediated cholesterol transfer across the mitochondrial membranes is not established, structural, biophysical, and biochemical studies have provided significant insight into this process. One study has shown that the processing of newly synthesized 37 kDa STAR is important for cholesterol transfer function while other reports support that association of the START domain with the outer mitochondrial membrane is sufficient to promote cholesterol transfer (Arakane et al. 1996, 1998, Artemenko et al. 2001, Bose et al. 2002, Baker et al. 2007).

Structural modeling and biophysical studies indicate that conformational changes, e.g. movement of the C-terminal α-helix, a pH-dependent molten globule transition, are important to promote cholesterol release and activate cholesterol transfer across the mitochondrial membranes (Bose et al. 1999, Baker et al. 2005, Yaworsky et al. 2005, Murcia et al. 2006, Barbar et al. 2009, Fluck et al. 2011). Cholesterol desorption into the intramembrane space for uptake by the inner membrane has been suggested for StAR functioning independent of cholesterol transfer (Christenson & Strauss 2001). However, more recent biochemical studies indicate functional interactions between StAR and components of a putative cholesterol transfer channel, suggesting a multi-protein complex transfer mechanism for cholesterol movement from the outer to the inner mitochondrial membrane (Hauet et al. 2005, Bose et al. 2008ab, Rone et al. 2009). There is evidence to suggest that StAR phosphorylation occurs at the mitochondria and that StAR phosphorylation is important for processing to the 30 kDa protein (Artemenko et al. 2001, Bose et al. 2008ab, Dyson et al. 2008). Once StAR has been imported into the mitochondrial matrix and processed to the 30 kDa mature form, it is no longer functional since it is no longer accessible to the mitochondrial outer membrane. A more in-depth description of these current models of StAR mechanism of action can be found in recent reviews (Miller 2007, Papadopoulos et al. 2007, Lavigne et al. 2010). Although a consensus for a mechanism for StAR-mediated cholesterol transport requires further study, all models are similar to the requirement for continual synthesis of the 37 kDa StAR in response to tropic hormone stimulation to maintain cholesterol transfer into the mitochondria.

Metastatic axillary lymph node protein 64 (MLN64) was identified by differential screening of a cDNA library for amplified products in breast cancer-derived MLN and was found to contain a domain that shared 33% sequence identity and 53% sequence similarity with the human StAR START domain (Tomasetto et al. 1995, Moog-Lutz et al. 1997). Thus, MLN64 was recognized as a START protein and named STARD3. STARD3/MLN64 is a transmembrane protein that is targeted to the late endosomes by an N-terminal MENTAL (MLN64-N-terminal) domain with a predicted membrane topology of four transmembrane helices that orients the C-terminal START domain facing the cytoplasm (Tomasetto et al. 1995, Alpy et al. 2001).

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).

**STARD1 and cholesterol transport to mitochondria**

**Acute regulation of steroidogenesis** Steroidogenic cells of the adrenal and gonads respond to tropic hormone stimulation by rapidly increasing the rate of steroid hormone biosynthesis. The first enzymatic reaction of steroidogenesis is the conversion of cholesterol to pregnenolone by the cholesterol side-chain cleavage complex, which is localized to the mitochondrial inner membrane. This step requires the delivery of the substrate cholesterol from cellular stores to the inner mitochondrial membrane, a process that occurs rapidly in response to tropic hormone stimulation and requires de novo protein synthesis. Thus, a protein that is synthesized in response to hormone stimulation and promotes cholesterol transfer...
movement to the mitochondrial matrix would be a candidate for the acute regulation of steroidogenesis (reviewed in Stocco 2001).

StAR was first described as a 30 kDa protein in hormone-treated rat adrenal cortex and cell culture systems as a protein induced by trophic hormone stimulation. Its expression pattern was correlated with increased steroid hormone output (Krueger & Orme-Johnson 1983, Pon et al. 1986a,b, Albert et al. 1989, Stocco & Chen 1991, Stocco & Sodeman 1991). Cloning the 30 kDa cDNA from the MA-10 mouse Leydig tumor cell line revealed a predicted protein of 284 amino acids and no sequence similarities at either the nucleic acid or the protein level within the databases (GenEMBL and SWISS-PROT, GCG Package, University of Wisconsin) indicating, at that time, that the 30-kDa protein represented a novel protein (Clark et al. 1994). As stated above, in heterologous transient transfection experiments, expression of the cDNA-encoded 30 kDa protein resulted in an increase in steroid synthesis, and so was named the StAR (Clark et al. 1994). To date, the vast literature on StAR (herein referred to as STARD1) provides strong biochemical and genetic data that support STARD1’s role in cholesterol transfer in regulated steroidogenesis (reviewed in Stocco 2001). Of particular importance was the finding that mutations in the hSTARD1 gene are the most common basis for congenital lipoid adrenal hyperplasia (lipoid CAH; Lin et al. 1995, Bose et al. 1996, King et al. 2011), a disorder characterized by the inability to synthesize adrenal or gonadal steroid hormones due to the absence of cholesterol transport into mitochondria. Recently, new mutations have been identified in the STARD1 gene that lead to partial loss of function and less severe lipid CAH (Fauchier et al. 2011). Star1 knockout mice confirmed that in the absence of the protein, the adrenal and gonads accumulated significant lipid deposits and the animals die shortly after birth due to the absence of adrenal hormones (Caron et al. 1997). Re-expression of a Star1 transgene in the knockout mice fully restored adrenal and gonadal steroidogenesis, as would be anticipated. However, mice that expressed an amino terminal truncated STARD1 that was not targeted to the mitochondria had partially restored steroidogenesis in a tissue- and gender-specific manner and retained lipid accumulation in the adrenal and gonads (Sasaki et al. 2008). These data support that STARD1 is capable of functioning without being targeted to the mitochondria but highlight the importance of correct and efficient subcellular localization of STARD1 for full function in vivo.

STARD1 and oxysterol production in non-steroidogenic tissues

In vitro protein overexpression studies demonstrated that STARD1 transports cholesterol across mitochondrial membranes in many cell types, potentially expanding STARD1 function outside of steroidogenic tissues (Sugawara et al. 1995). One of the first reported roles for STARD1 outside of steroidogenic cells was in cholesterol transfer across the mitochondrial membranes in the liver for initiation of bile acid synthesis by the alternative pathway. Overexpression of STARD1 significantly increases 27HC and bile acid synthesis in primary rat or mouse hepatocytes or human HepG2 hepatoma cells (Pandak et al. 2002, Ren et al. 2004a,b, Hall et al. 2005). Enhanced rates of bile acid synthesis also occur in both rats and mice after overexpression of STARD1 in the liver, providing evidence for an in vivo function (Ren et al. 2004a,b). A key finding of these studies is that the transport of cholesterol into the mitochondria is rate-limiting for bile acid synthesis by the CYP27A1 alternative pathway, suggesting that cholesterol transport into hepatic mitochondria may be regulated under normal or pathological conditions (Pandak et al. 2002). Importantly, STARD1 expression in human HepG2 hepatoma cells is increased by treatment with 27HC or by induced expression of CYP27A1, the enzyme that metabolizes cholesterol to 27HC. LXR-α-dependent transactivation of Star1 has been established in mouse adrenocortical cells (Cummins & Mangelsdorf 2006, Cummins et al. 2006). Therefore, 27HC oxysterol activation of LXR-α may account for the observed increase in STARD1 in HepG2 cells. Strikingly, overexpression of Star1 in liver of ApoE-deficient mice improved serum and liver lipid profiles and reduced lipid accumulation in aortic segments (Ning et al. 2009a,b). It has been proposed that the anti-atherogenic action of hepatic STARD1 expression is due to both increased oxysterol synthesis leading to LXR-mediated anti-atherogenic effects and increased bile acid synthesis leading to clearance of cholesterol (Ning et al. 2009a,b). Thus, mechanisms to enhance STARD1 expression in hepatocytes have been proposed as a target to help attenuate dyslipidemia and the development of atherosclerosis (Ning et al. 2009a,b). However, a potential beneficial role for STARD1 expression in hepatocytes is complicated by recent findings that show elevated STARD1 levels in liver are associated with non-alcoholic fatty liver disease (NAFLD) and hepatocellular carcinoma (discussed below).

STARD1 overexpression in human THP-1-derived macrophages also decreases total lipid and cholesterol levels (Bai et al. 2009, Ning et al. 2009a,b). The LXR-α-dependent target genes ABCG1, PPARγ, LXRα, and CYP27A1 are increased at the mRNA and protein levels in the STARD1 overexpressing cells. Treatment of THP-1 cells with the oxysterols 25HC or 27HC had similar effects on gene expression as STARD1 overexpression. These data suggest STARD1 overexpression functions in macrophages to supply cholesterol for CYP27A1-dependent oxysterol synthesis that promotes LXR-α-dependent mechanism(s) to decrease cholesterol levels (Ning et al. 2009a,b). In support of this model, stable overexpression of STARD1 in RAW264.7 murine macrophages resulted in increased 27HC levels (Taylor et al. 2010). STARD1 overexpression also increased LXR-α expression, LXR-α-dependent reporter gene activity, ABCA1 expression, and cholesterol efflux, similar to STARD1 functioning within the LXR pathway. Suppression of the SREBP2 pathway by 27HC was indirectly indicated in these studies by decreased expression of the target genes.
HMGR and LDLR (Taylor et al. 2010). Interestingly, overexpression of STARD1 in THP-1 macrophage cells attenuates ox-LDL-induced inflammatory cytokine release and apoptosis (Ning et al. 2009a,b). It remains to be determined whether this effect is mediated by elevated oxysterols generated in response to STARD1 overexpression.

Detection of STARD1 protein in liver has not been consistently reported and most of the functional link to bile acid synthesis has been proposed in systems where STARD1 is exogenously supplied. Therefore, it is not clear whether endogenous STARD1 expression in the liver is sufficient to contribute significantly to bile acid synthesis via the alternative pathway. There are no reports of either bile acid disorders or increased risk for cardiovascular disease in patients with lipid CAH who lack a functional STARD1 protein, suggesting that STARD1 is not required for liver or macrophage cholesterol metabolism. However, this does not exclude the possibility that another START protein functions in this process or that aberrant overexpression of STARD1 may occur in pathological states (see below). An important question to address is whether STARD1 levels are induced in hepatocytes and macrophages in pathological states and to determine the mechanism(s) of regulation.

**STARD1 in fatty liver disease**

One example for increased STARD1 expression in disease states may be in NAFLD. NAFLD describes a group of disorders associated with an accumulation of lipids, mostly triacylglycerol, in the liver. NAFLD is a common consequence of obesity and type 2 diabetes mellitus that can lead to non-alcoholic steatohepatitis (NASH) and states of hepatic fibrosis and cirrhosis. The potential for cholesterol metabolism disorders in NAFLD disease progression is suggested by reports that hepatic cholesterol accumulation enhances the progression of NAFLD to NASH in mouse models (Van Rooyen et al. 2011). A similar result appears in humans where free cholesterol levels in the liver are elevated in patients with NASH relative to patients with NAFLD or without fatty liver disease (Puri et al. 2007, Caballero et al. 2009). HMGR and SREBP2 transcript levels in liver were elevated in patients with NAFLD and NASH, providing a rationale for the elevated hepatic free cholesterol levels (Caballero et al. 2009). In this cohort, STARD1 mRNA was also increased in the liver with levels being highest in NASH patients. These data indicate a possible positive correlation between STARD1 expression and hepatic cholesterol levels, although STARD1 protein levels in the NAFLD and NASH patients remain to be measured. SREBP2 and LDLR protein levels were increased and ABC transporters were decreased in an obese, diabetic mouse model that develops NASH when on a high-fat diet (Van Rooyen et al. 2011), and the authors proposed a central role for SREBP2 in disease progression (Van Rooyen & Farrell 2011). The lipotoxicity associated with increased hepatic cholesterol levels and disease progression from NAFLD to NASH has been proposed to be mediated at the level of mitochondria (reviewed in Montero et al. 2010). Current data indicate that mitochondria are more susceptible to apoptotic stimuli due to glutathione depletion resulting in increased reactive oxygen species levels (Mari et al. 2006). Thus, the possible positive association for STARD1 and cholesterol in NASH patients would suggest a mechanism for increased mitochondrial cholesterol content. It would be interesting to examine additional START protein family members in this disease. In particular, STARD4, which is highly expressed in hepatocytes and is regulated by SREBP2, is a strong candidate for a START protein involved in fatty liver disease (see below).

**STARD1 and hepatocellular carcinoma**

An increase in de novo cholesterol synthesis in hepatocellular carcinomas has been a long-standing observation (Siperstein & Fagan 1964) and only recently studies have begun to address the potential mechanism(s) for increased cholesterol synthesis in liver cancers. As with NASH, one focus has been on mitochondria with early observations that mitochondria isolated from hepatoma xenografts have increased cholesterol:phospholipid ratio relative to mitochondria from normal rat liver (Feo et al. 1973, Crain et al. 1983). Increased mitochondrial cholesterol content in cancer cells has the potential for suppression of apoptosis by decreasing mitochondrial permeability and suppressing cytochrome c release. Mitochondrial permeability is regulated by a multi-protein complex that spans the inner and outer mitochondrial membranes termed the mitochondrial permeability transition pore (mPTP; Henry-Mowatt et al. 2004). The major components of the mPTP include the voltage-dependent anion channel, the adenine nucleotide translocase, and cyclophilin D (Alirol & Martinou 2006). Apoptosis can be induced by activation of either the extrinsic or intrinsic apoptotic pathways and activation of either pathway ultimately results in caspase activation leading to cell death (Riedl & Salvesen 2007). The intrinsic apoptotic pathway involves disruption of the mPTP resulting in release of cytochrome c and pro-apoptotic proteins into the cytoplasm (Henry-Mowatt et al. 2004). Expression of pro-apoptotic BCL2 proteins, BAX and BAK, forms homo-oligomers that insert into the mitochondrial outer membrane and disrupt mPTP leading to release of cytochrome c (Cory & Adams 2002).

Elevated mitochondrial cholesterol content in hepatocellular carcinoma has recently been linked to increased STARD1 expression. Rat H35 and human HepG2 hepatoma cell lines and human hepatocarcinoma samples have elevated mitochondrial cholesterol content relative to cholesterol content of normal rat and human liver (Montero et al. 2008). The cholesterol levels within these cell lines correlated with an increase in SREBP2 expression (Montero et al. 2008). Blocking cholesterol synthesis in the HepG2 cells increased sensitivity to agents that induce mPTP and apoptosis, indicating a link between mitochondrial...
cholesterol content and chemoresistance. Cholesterol loading of isolated rat liver mitochondria increased membrane order and suppressed BAX-mediated release of cytchrome c, supporting the concept that cholesterol-enriched mitochondria are more resistant to apoptosis. STARD1 protein was highly expressed in the HepG2 cells and siRNA-mediated knockdown of STARD1 resulted in decreased mitochondrial cholesterol content and increased sensitivity to apoptosis-inducing agents. Thus, in hepatocellular carcinoma, overexpression of SREBP2 and increased cholesterol levels together with aberrant increased expression of STARD1 in the tumor may provide a mechanism for elevated mitochondrial cholesterol levels and increased resistance to apoptosis.

**STARD3 and lysosomal cholesterol**

STARD3/MLN64 is a transmembrane protein localized to the late endosomes by an N-terminal MENTAL (MLN64–N-terminal) domain with the C-terminal START domain facing the cytoplasm (Table 1; Tomasetto et al. 1995, Alpy et al. 2001). The location of STARD3/MLN64 to late endosomes led to studies on its potential role in Niemann Pick type C disease. Niemann Pick type C disease is a lipid storage disorder caused by mutations in genes encoding either NPC1 or NPC2 that result in accumulation of cholesterol in lysosomal storage organelles, which leads to neurological disorders and hepatosplenomegaly (reviewed in Rosenbaum & Maxfield 2011). In brief, free cholesterol that is generated by hydrolysis of LDL-derived cholesterol esters is bound by NPC2 (Niemann-Pick C2), a soluble late endosomal/lysosomal luminal protein, and transferred to the N-terminal cholesterol-binding domain of the late endosome transmembrane protein NPC1 (Niemann-Pick C1). NPC1 then transfers cholesterol across the membrane for release from the lumen by an undefined mechanism (Wang et al. 2010). The MENTAL domain of STARD3 is capable of binding cholesterol and is required for its dimerization with another endosomal membrane protein composed only of a MENTAL domain termed MENTHO (MLN64 N-terminal homolog; Alpy et al. 2005). In one model for trafficking of late endosome/lysosome cholesterol, STARD3/MLN64 acts as the cytosolic acceptor of NPC1-derived cholesterol (reviewed in Strauss et al. 2003). Alternatively, STARD3/MLN64 and MENTHO may bind cholesterol via the MENTAL domains and independently move cholesterol across the membrane (Alpy et al. 2005, Alpy & Tomasetto 2006, Charman et al. 2009). The fate of cytosolic cholesterol bound by STARD3/MLN64 may be direct absorption by a closely associated membrane or transfer to another soluble cholesterol-binding protein, potentially a member of the STARD4 subfamily (Soccio & Breslow 2003, Alpy & Tomasetto 2006). However, it is not clear whether STARD3/MLN64 is required for cholesterol trafficking in vivo (Kishida et al. 2004). Homozygous STARD3/MLN64 mutant mice that express a STARD3/MLN64 protein containing the N-terminal MENTAL domain but lacking the START domain do not accumulate cholesterol in late endosomes/lysosomes and synthesize steroid hormones at wild-type levels (Kishida et al. 2004). STARD4, STARD5, Npc1, and Npc2 mRNA levels were not changed due to loss of the START domain from STARD3/MLN64, suggesting that the lack of a phenotype was not due to compensatory increases of these cholesterol transporters. However, whether the intact MENTAL domain of STARD3/MLN64 may be responsible for the function of this transporter in late endosomes in the knockout mice, or whether STARD4 or STARD5 can act as the soluble cytoplasmic acceptor of cholesterol from STARD3/MLN64 or NPC1, remains to be determined (Fig. 1).

**STARD4 subfamily: the soluble sterol-binding proteins**

The STARD4 subfamily is composed of STARD4, STARD5, and STARD6 and is most closely related to the STARD1/D3 subfamily with ~20% sequence identity (Soccio et al. 2002; Table 1). STARD4 was identified as a novel EST in a cDNA microarray study designed to identify cholesterol-regulated genes in mouse liver (Soccio et al. 2002). Mice fed a high-cholesterol diet had reduced Stad4 transcript levels with Stad4 gene expression later shown to be regulated by a SREBP2-dependent mechanism (Soccio et al. 2002, 2005, Rodriguez-Agudo et al. 2011). STARD5 and STARD6 were identified from a BLAST search of the human genome against STARD4 (Soccio et al. 2002). Analysis of deduced amino acid sequences for the STARD4 family predicts ~22 kDa soluble proteins entirely composed of the START domain and lacking any membrane targeting sequence (Table 1). The soluble cytoplasmic localization for these STARD4 proteins has generated much speculation on their role in cholesterol trafficking.

As outlined above, cholesterol transport to mitochondria can result in oxysterol synthesis for bile acid metabolism or LXRα-dependent responses depending on the tissue and cell type. START proteins that function to traffic cholesterol to the ER would enhance ER cholesterol that would increase ACAT activity and cholesterol ester synthesis and potentially suppress SREBP2 processing leading to decreased cholesterol synthesis. Alternatively, accumulation of cholesterol in the ER may promote ER stress. All of these endpoints have been attributed to START proteins of this subfamily; the question is which START protein plays a physiological or pathological role in which cell type and under what conditions? To explore possibilities for the role of this subfamily of START proteins in cholesterol transport, the similarities and differences between the members are reviewed with a focus on the current proposed functions.

**STARD4 and cholesterol transport to the mitochondria and ER**

The potential for STARD4 to deliver cholesterol to both mitochondria and ER is based on several studies where
STARD4 is overexpressed in cell culture systems. Heterologous expression of STARD4 in COS-1 cells or addition of recombinant purified STARD4 to isolated mitochondria stimulated cholesterol transfer into mitochondria, although with lower efficiency relative to STARD1 (Soccio et al. 2005, Bose et al. 2008a,b). STARD4 overexpression in primary mouse hepatocytes increased bile acid synthesis and cholesterol ester synthesis (Rodriguez-Aguado et al. 2008), indicating increased cholesterol transport to mitochondria and ER (Fig. 1). Since the expression of CYP7A1, the enzyme that regulates the classical bile acid synthesis pathway, is lost in cultured primary mouse hepatocytes (Hylemon et al. 1992), these data suggest that STARD4 has the capability to increase cholesterol transport to mitochondria for the alternative pathway for bile acid synthesis. Importantly, endogenous STARD4 protein was detected in human liver by immunohistochemistry and was shown to be expressed in hepatocytes and Kupffer cells, i.e. macrophages within the liver reticuloendothelial system (Rodriguez-Aguado et al. 2011). Endogenous expression in mouse 3T3-L1 fibroblasts and human THP-1 macrophages was repressed by sterol treatment and induced by blocking cholesterol synthesis by treatment with a HMGR inhibitor. These data are consistent with a SREBP2-mediated regulation for STARD4 in vivo (Soccio et al. 2005). STARD4 colocalized with ER marker protein calnexin in 3T3-L1 cells and with ACAT1 in THP-1 macrophages, an association that was more pronounced after treatment with HMGR inhibitors to increase SREBP2-dependent increase in STARD4 (Rodriguez-Aguado et al. 2011). Cholesterol ester synthesis was increased by addition of recombinant, purified STARD4 to isolated microsomes, indicating that STARD4 positively affects ACAT activity in vitro. The association of STARD4 with ER membranes and ACAT1 and the direct effect on ACAT activity strongly support STARD4 functions to transport cholesterol to the ER and ACAT1 for cholesterol ester synthesis (Fig. 1). It remains to be determined whether STARD4-mediated cholesterol transport to the ER may also play a role in providing substrate for CYP7A1 and enhancing the classical pathway for bile acid synthesis in hepatocytes.

A role for STARD4 functioning at the ER is further supported by a recent study that demonstrated STARD4 overexpression in U2OS osteosarcoma cells enhances the transport rate of a fluorescent cholesterol analog, DHE, to the endosome recycling complex and the ER. However, the cholesterol redistribution in U2OS cells mediated by STARD4 can be mimicked with injection of the non-specific cholesterol-binding compound, methyl-β-cyclodextrin, suggesting that STARD4 may contribute to non-selective sterol transport that is required to maintain proper cholesterol distribution between cellular membranes (Mesmin et al. 2011). STARD4 overexpression also increased cholesterol ester levels and the responsiveness of SCAP-SREBP2 processing and trafficking to changes in cellular cholesterol levels (Mesmin et al. 2011). The authors propose that STARD4 represents a component of the cellular cholesterol sensing system wherein STARD4 would transport cholesterol to the ER membrane and help modulate the SREBP2 pathway and ACAT1 activity (Mesmin et al. 2011).

STARD4-mediated cholesterol transport to the ER may also promote ER stress. Although Stard4/STARD4 is established as a SREBP2 target gene (Soccio et al. 2005, Rodriguez-Aguado et al. 2011), it is also an ER stress response gene. STARD4 mRNA was increased in HeLa cells between 2 and 6 h treatment with the ER stress inducer tunicamycin and returned to control levels between 12 and 24 h (Yamada et al. 2006). Reporter gene activity assays identified an ATF6-dependent responsive element, confirming that STARD4 promoter can be activated by transcription factors that are activated during the ER stress response. The significance for the transient STARD4 mRNA expression during ER stress is not known, but an increase in STARD4 protein expression in disease states associated with dyslipidemia and ER stress may impact cholesterol homeostasis by dampening the ER cholesterol sensing system and promoting cholesterol ester formation.

Surprisingly, homozygous STARD4 knockout mice do not present with a strong lipid phenotype; the plasma and hepatic lipid content for both male and female STARD4 null mice is comparable to its wild-type counterparts (Riegelhaupt et al. 2010). However, female STARD4 knockout mice have decreased cholesterol and phospholipid content in gallbladder bile. Lipid profiles compared after 1 week on diets supplemented with high cholesterol revealed elevated plasma and hepatic lipids as expected, but the female STARD4 knockout mice had ~20% lower plasma total cholesterol and cholesterol ester levels relative to the wild-type female mice with no differences in hepatic cholesterol, cholesterol ester, or triacylglycerol levels. There was no difference in the effect of lovastatin, a HMGR inhibitor that blocks cholesterol synthesis, on plasma or hepatic lipid profiles between the wild-type and STARD4 knockout mice. Stad5 and Stad3 mRNA expression appear to be repressed, although not significantly decreased, and Stad1 undetectable in the liver of STARD4 knockout mice, suggesting that the other major START proteins are not responsive to loss of STARD4. It remains to be determined whether environmental, dietary, or disease stressors may highlight a phenotype resulting from loss of STARD4 on cholesterol ester or bile acid synthesis.

STARD5 and cholesterol transport to the ER and PM

STARD5 does not transfer cholesterol to mitochondria in vitro (Bose et al. 2008a,b) and transient overexpression in primary mouse hepatocytes has no effect on bile acid synthesis rates (Rodriguez-Aguado et al. 2005), indicating that STARD5 does not function to transport cholesterol to mitochondria. Overexpression of human STARD5 in primary rat hepatocytes, however, resulted in increased cellular-free cholesterol levels with possible increased ER cholesterol content (Rodriguez-Aguado et al. 2005).
The redistribution of cholesterol was measured as a threefold increase in cholesterol recovered with microsomes isolated from STARD5 overexpressing cells compared with controls. STARD5 binds both cholesterol and 25HC (Soccio et al. 2002, Rodriguez-Agudo et al. 2005, 2008) and is expressed predominantly in liver and kidney (Soccio et al. 2002, Chen et al. 2009). In liver, STARD5 is localized to the Kupffer cells and is not expressed in hepatocytes (Rodriguez-Agudo et al. 2006). This observation was confirmed by subcellular localization studies for STARD5 protein in cell lines from human macrophages and monocytes as well as mast, lymphoblast, and promyeloblast cells. Double immunofluorescence studies in human THP-1 macrophages revealed that STARD5 was localized to the perinuclear regions of the cell and colocalized with Golgi but not with endosome markers (Rodriguez-Agudo et al. 2006). Filipin staining to detect distribution of free cholesterol in the macrophages revealed high cholesterol concentration within the Golgi, suggesting localization of STARD5 with membranes enriched in free cholesterol. In mouse kidney sections, STARD5 protein was detected by immunohistochemistry in the proximal tubules, but not in the glomeruli (Chen et al. 2009). The staining pattern indicated diffuse cytoplasmic distribution with concentrated expression at the apical membrane. Greater resolution for STARD5 subcellular distribution by immunoelectron microscopy confirmed diffuse cytoplasmic distribution with enriched staining along the brush-border (apical) and rough ER membranes with no apparent association with mitochondria or Golgi apparatus. In HK-2 human proximal tubule cells, double immunofluorescence confocal microscopy showed that STARD5 had a punctate expression pattern that colocalizes with the ER but not endosome marker proteins. Together, the data indicate a potential broad cellular distribution, e.g. cytoplasm–PM–Golgi–ER, for STARD5 in macrophages and renal proximal tubules (Fig. 1). As a soluble sterol transporter, STARD5 may shuttle cholesterol between the Golgi, ER and PM, although STARD5’s trafficking remains to be determined. However, unlike STARD4, STARD5 overexpression does not increase cholesterol ester synthesis rates (Rodriguez-Agudo et al. 2005, 2008) or ACAT activity (Rodriguez-Agudo et al. 2011), indicating a distinction for the fate of cholesterol transported to the ER by these two lipid transporters. Another distinction in overexpression systems is that STARD5 promotes an increase in free cholesterol levels while STARD4 has no effect on free cholesterol levels (Rodriguez-Agudo et al. 2005, 2008). In human proximal tubule cell lines, STARD5 expression is higher in the cells with greater cholesterol content, supporting a positive correlation between cellular free cholesterol content and STARD5 expression in the kidney (B J Clark, unpublished observations). STARD5 overexpression in THP-1 macrophages markedly increases SREBP2 mRNA levels, suggesting a potential for increased cholesterol synthesis (Borthwick et al. 2010). Therefore, STARD5 may contribute to determining the levels of cellular free cholesterol content.

STARD5/STARD5 mRNA expression is increased by agents that promote ER stress, such as in thapsigargin-treated NIH-3T3 and HK-2 cells or cholesterol-loaded mouse macrophages (Soccio et al. 2005, Chen et al. 2009). In HK-2 human proximal tubule cells, chemically induced ER stress promotes STARD5 redistribution from a diffuse to a more prominent perinuclear and cell membrane localization (Chen et al. 2009). The role for STARD5 during ER stress is not known but chronic ER stress and inflammation are underlying metabolic disorders in many disease states, including NAFLD, type II diabetes, and cancer (Tsai & Weissman 2010, Malhi & Kaufman 2011). There is one study looking at STARD5 expression in disease states associated with ER stress. In a diabetic mouse model, STard5 steady-state mRNA and STARD5 protein levels in kidney were shown to be significantly increased, as were free cholesterol levels, compared with wild-type control mice (Chen et al. 2009). Cholesterol accumulation in the ER is known to promote ER stress; however, the significance of the association between elevated renal cholesterol, ER stress, and STARD5 in diabetic kidney remains to be determined.

In summary, the differential regulation and distinct cell-type distribution help to control potential redundant actions of STARD4 and STARD5. STARD4 is expressed in hepatocytes and regulated by SREBP2 and activates ACAT. Therefore, in hepatocytes, STARD4 is a strong candidate for cholesterol transport to mitochondria for bile acid synthesis and to the ER for cholesterol ester synthesis. Both STARD4 and STARD5 are expressed in macrophages and both may function as cholesterol transporters that shuttle cholesterol to the ER. STARD4 would increase ACAT and cholesterol ester synthesis while STARD5 may promote an increase in free cholesterol level resulting in ER stress. Both STARD4 and STARD5, therefore, may promote foam cell development and increase the risk for atherosclerosis. Alternatively, STARD5 may serve as a cholesterol buffer to bind the free cholesterol and help suppress the potential lipotoxicity of excess free cholesterol in the cell. Similar functions proposed for STARD5 in macrophage would apply to renal proximal tubule cells. In addition, the prominent apical membrane localization in polarized epithelial cells of renal proximal tubules indicates a potential role for STARD5 in PM cholesterol (Fig. 1). Whether STARD5 contributes to lipid raft formation and stabilization or conversely, extraction of PM cholesterol remains to be determined. Finally, STARD5 binds 25HC and its role in oxysterol transport has yet to be examined.

STARD6 and cholesterol transport to the mitochondria

STARD6 was originally shown to be predominantly expressed in mouse testes and later specifically localized in rat testis to the germ cells with highest expression in round spermatids (Soccio et al. 2002, Gomes et al. 2005). The function of STARD6 in spermatogenesis is not known. However, STARD6 was recently identified as a putative gene
required for mitochondrial NADH-dependent dehydrogenase activity (diaphorase) associated with sperm motility and quality (Golas et al. 2010). Using recombinant inbred mice strains, a quantitative trait loci approach identified three chromosomal regions, 19q43–19q47, 18q44, and 18q49–18q50, that segregated with diaphorase activity (Golas et al. 2010). Star6 was highlighted as a putative gene within 18q44, leading to the speculation that Star6 along with other genes may regulate activity of a mitochondrial enzyme. The significance of this observation may be linked to earlier work that showed addition of recombinant purified Star6 to isolated mitochondria-stimulated cholesterol transfer as efficiently as the START domains of Star1 and Star3 (Bose et al. 2008a,b). Furthermore, Star6 protein folding, cholesterol binding, and association with the mitochondrial outer membrane are all very similar to Star1, suggesting that this protein may function at the mitochondrial level in male germ cells (Bose et al. 2008a,b). Similar to other members of the Star4 subfamily, Star6 lacks any organelle targeting sequence. To validate potential actions for Star6 at the mitochondria, future studies are required to determine the subcellular localization of Star6 in male germ cells. Expression has also been reported in rat brain and nervous system with potential regulation under neurotoxic conditions (Chang et al. 2009).

The phospholipid/sphingolipid-binding START proteins

**STAR2/PCTP subfamily: the phosphatidylcholine and ceramide transporters**

This subfamily is composed of Star2, Star7, and Star10 that all bind phosphatidylcholine (PC) and Star11 that binds ceramide (Table 1). Star2/PCTP was purified from bovine liver and was shown to exchange specifically PC within a membrane or to shuttle PC from the ER to the PM. The crystal structure of Star2 with bound PC shows the classical helix grip fold that forms a large hydrophobic tunnel. The choline head group provides the specificity of binding and the binding pocket can accommodate PC with saturated or unsaturated acyl groups of different lengths (Roderick et al. 2002).

Star7 (also referred to as gestational trophoblastic tumor gene–1, GTTI) was first identified as a transcript that was overexpressed in JEG-3 choriocarcinoma cells (Durand et al. 2004). Star7 shares 25% sequence identity with Star2/PCTP and the purified protein extracts PC but not phosphatidylserine, phosphatidylethanolamine, or sphingomyelin from lipid vesicles in vitro (Horibata & Sugimoto 2010).

Star10 was identified as a 35 kDa anti-phospho-FKHR immunoreactive band that was overexpressed in tumors of ErbB2 transgenic mice (Olayioye et al. 2004). Recombinant purified Star10 specifically extracts PC and PE from reconstituted lipid vesicles and enhances PC and PE transfer from donor to acceptor vesicles in vitro (Olayioye et al. 2005). In vitro, PC and PE are recovered by immunoprecipitation of overexpressed Star10 in HEK-293 cells. In both the in vitro and in vivo studies, PC appears to be the preferred lipid for Star10 (Olayioye et al. 2005). Phosphorylation of Star10 on Ser284 by casein kinase II decreases in vitro lipid transport activity, possibly by decreasing membrane association (Olayioye et al. 2007).

Star11 is more commonly known as CERT, a ceramide transport protein shown to be a splice variant of the Goodpasture antigen-binding protein (GBPAP26). Within this subfamily, Star2/PCTP and Star11/CERT have been studied in more detail and have been recently reviewed by others (Kanno et al. 2007a,b, Hanada et al. 2009, Kang et al. 2010, Mencarelli et al. 2010). Therefore, only summaries of the recent data on the members of this subfamily are provided.

**STAR2/PCTP and insulin resistance**

The initial proposed functions for PCTP were in PC transport across the hepatic canalicular membrane and for lung surfactant synthesis, given that PC is the major phospholipid in bile and surfactant and the transporter is expressed in the hepatocytes and alveolar cells (van Helvoort et al. 1999). However, PCTP knockout mice (Pctp−/−) have no apparent phenotype with normal levels of PC measured in the bile and lung surfactant (van Helvoort et al. 1999). Although the predicted phenotype(s) was not observed with the Pctp−/− mice, new data indicate Star2/PCTP functions in insulin-regulated pathways to maintain glucose homeostasis (Scapa et al. 2008, Shishova et al. 2011). Fasting serum glucose and free fatty acid levels are significantly decreased in Pctp−/− mice compared with wild-type counterparts due to increased insulin sensitivity (Scapa et al. 2008). In addition, hepatic SREBP1c expression is decreased in Pctp−/− mice along with downstream target gene expression within the fatty acid biosynthesis pathway. Isolated hepatocytes from Pctp−/− mice have decreased fatty acid synthesis rates, providing a functional readout to support the gene expression profile. Treatment of wild-type mice with a Star2/PCTP small molecular inhibitor (compound A1) that displaces PC binding attenuates high-fat diet-induced increase in serum glucose levels (Shishova et al. 2011). Treatment of human hepatocytes and HEK-293 cells with compound A1 promotes activation of the insulin signaling pathway (Shishova et al. 2011). Together, the data indicate that blocking Star2/PCTP function in the liver results in increased hepatic insulin sensitivity. The mechanism for Star2/PCTP action in liver glucose metabolism, however, remains to be determined.

Star2/PCTP was recently shown to interact with thioesterase superfamily member 2 (Them2) and the transcription factor paired box gene 3 (PAX3; Kanno et al. 2007a,b). The significance of these particular protein–protein

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interactions is that other START family members, including mammalian STARD14 and STARD15, are multi-domain proteins that have amino-terminal thioesterase domains. In plants, a major START protein subfamily is characterized by homeobox domain(s) and function in DNA-binding and transcriptional regulation (Schrick et al. 2004). Interestingly, the thioesterase activity of recombinant Them2 in in vitro assays is enhanced by the presence of STARD2 as is the transactivation activity of PAX3 (Schrick et al. 2004). It has been speculated that during cold adaptation in mice, PCTP–Them2 interaction in brown fat increases and attenuates the uptake and oxidation of fatty acids within mitochondria (Kang et al. 2009, 2010). Although the mechanism(s) are not defined, it has been proposed that phosphorylation of STARD2/PCTP results in mitochondrial association and interaction with Them2. The PCTP–Them2 interaction increases Them2 activity resulting in a decrease in fatty acyl-CoA levels and thereby a decrease in fatty acid oxidation (Kang et al. 2009, 2010).

STARD7: a phosphatidylcholine-binding protein

A STARD variant, STARD7-1, was identified by a BLAST search and shown to have an extended amino terminal sequence that forms an amphipathic helix that functions as a mitochondrial targeting sequence (Horibata & Sugimoto 2010). Mitochondrial PC levels in the HEPA-1 mouse hepatoma cell line are increased after STARD7 overexpression but are unchanged by STARD7 silencing by siRNA. STARD7-1 can be processed to a smaller protein, presumably by mitochondrial proteases that cleave the targeting sequence, but it remains sensitive to protease digestion when isolated mitochondria are treated with proteinase K (Horibata & Sugimoto 2010). Thus, STARD7 appears to remain on the cytoplasmic side of the mitochondrial outer membrane. This membrane association would be consistent with both the cytosolic and mitochondrial localization of endogenous STARD7-1 in the HEPA-1 mouse hepatoma cell line and rat liver. Regulating mitochondrial PC levels would influence membrane structure and, as the authors of this study speculate, regulate acylation reactions (Horibata & Sugimoto 2010). Since STARD7 is expressed at relatively high levels in lung, colon, and liver cancer cell lines (Durand et al. 2004), it may play a more general role in proliferating cells, possibly for supply of PC for mitochondrial biogenesis.

STARD10: a phosphatidylcholine/ethanolamine-binding protein in breast cancer

STARD10 was originally reported to be co-expressed with ErbB2/HER2/neu in breast cancer cell lines and primary breast carcinomas (Olayioye et al. 2004). Functionally, the overexpression of STARD10 in NIH-3T3 fibroblast cells promoted anchorage-independent cell growth only if expressed together with ErbB2, suggesting that STARD10 may function within the ErbB2/HER2/neu receptor signaling pathway (Olayioye et al. 2004). However, STARD10 and HER2/neu mRNA and protein expression levels were later shown to be inversely correlated when analyzed in breast tumors from a large cohort of patients (Murphy et al. 2009). Unexpectedly, 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 (Murphy et al. 2009). Whether the beneficial effect for STARD10 expression in breast cancer is related to its PC binding/transport activity will require elucidating the biological functions of STARD10 in mammary tissues (Olayioye et al. 2005). This function most likely will be related to the phosphorylation state of STARD10, therefore elucidating the phosphatase(s) responsible for the dephosphorylation and activation of STARD10, and the pathways involved in STARD10 activation are important future studies (Olayioye et al. 2007).

STARD11/CERT: a ceramide-binding protein

STARD11 is unique within this subfamily in that the protein contains additional motifs that localize the START domain to its cellular sites of action. STARD11/CERT is responsible for the movement of ceramide from the ER to the Golgi membrane (Hanada et al. 2003). The protein has an amino terminal pleckstrin homology domain (PH), a middle region with a FFAT motif, and carboxyl terminal START domain (Table 1). The PH domain binds to phosphoinositides, specifically PI4P in the Golgi membrane, while the FFAT motif interacts with the ER resident protein VAP. STARD11/CERT phosphorylation is proposed to maintain the protein in a folded, inactive form with dephosphorylation resulting in a conformational change that exposes the PH and FFAT domains for membrane interaction and positions the START domain for ceramide transfer. A detailed description of the proposed model for ceramide transfer by STARD11/CERT has been presented (Hanada et al. 2009) and the basic concept is the orientation of the protein with the N-terminus bound to the Golgi membrane and the middle region bound to the ER would place the START domain in close proximity to both membranes to facilitate ceramide extraction from the ER and delivery to the Golgi. The crystal structure of the STARD11/CERT START domain confirmed the helix–grip fold structure for ceramide binding and supports a mechanism for membrane interaction and ceramide extraction/absorption (Kudo et al. 2008, 2010).

The multi-domain START proteins

STARD8/12/13: the SAM-RhoGAP-START subfamily

This subfamily is more commonly referred to as the deleted in liver cancer (DLC) family of proteins. The history, genomic structure, isoform expression, and known and potential
function(s) for STARD12/DLC-1, STARD13/DLC-2, and
STARD8/DLC-3 have been reviewed (Durkin et al. 2007a,b)
and only a few aspects are highlighted here. Members of this
subfamily share the same multi-domain structure, an amino
terminal sterile α motif (SAM; Ponting 1995) followed by a
serine-rich region, a RhoGAP domain, and a carboxy-
terminal START domain (Table 1). STARD12/DLC-1 was
first isolated as a genomic clone that was localized on
chromosome 8p21.3–22, a region associated with loss of
heterozygosity in several cancers and shown to be deleted in
50% of primary human hepatocellular carcinoma tumor
tissues (Yau et al. 1998). Re-expression of DLC-1 in human
liver, lung, breast, and ovarian cancer cell lines suppresses cell
growth and increases apoptosis, supporting DLC-1 as a tumor
suppressor. Protein kinase D phosphorylates multiple sites on
STAR 12/DLC-1 and the phosphoprotein has decreased
activity (Scholz et al. 2009, 2011), suggesting a potential
regulatory mechanism controlling STARD12/DLC-1 func-
tion. Targeted deletion of St ard 12/dlc-1 gene in mice results in
embryonic lethality, most likely due to disruption of
cytoskeletal organization. STARD12/DLC-1 has been
shown to regulate RhoA activity via the RhoGAP domain,
tocolocalize with focal adhesions via binding to the SH2
domain of tensin 1, and to stimulate PLC-β1 leading to IP3-
dependent intracellular Ca2+ release (Durkin et al. 2007a,b).
Any or all of these functions could disrupt/promote
cytoskeletal organization.

STARD13/DLC-2 and STARD8/DLC-3 also have
tumor suppressor activities when overexpressed in cancer
cell lines (Ching et al. 2003, Durkin et al. 2007a,b) and localize
to focal adhesions (Kawai et al. 2007, 2009), indicating similar
activities as characterized for STARD12/DLC-1. However,
STARD13/DLC-2 and STARD8/DLC-3 cannot compen-
sate for loss of STARD12/DLC-1. Recent reports character-
ing STARD13/DLC-2 knockout mice show that the mice
are healthy and fertile with no overt phenotype (Yau et al.
2009, Lin et al. 2010). The knockout mice were not more
susceptible to spontaneous tumors or induced hepatocarci-
nogenesis, indicating potential compensatory effects of the
other DLCs for tumor suppressor activity or possible require-
ment for a ‘second hit’ to promote tumor formation
(Yau et al. 2009, Lin et al. 2010). However, STARD13/DLC-
2 may help suppress angiogenesis associated with tumor
growth (Lin et al. 2010). STARD13/DLC-2 has widespread
tissue distribution, with expression observed in CD31-
positive cells of blood vessels, indicating endothelial specific
expression. Based on this observation, matrix-released
vascularization and B16 murine melanoma xenograft tumor
cell growth assays were performed and angiogenesis was
shown to be enhanced in the knockout mice compared with
wild-type counterparts. Silencing STARD12/DLC-2
expression in HUVECs leads to increased cell migration in
a RhoA-dependent manner, supporting the in vivo data for
STARD12/DLC-2 promoting angiogenesis (Lin et al. 2010).

To date, the functions associated with this subfamily have
been attributed to the RhoGAP domain. The role of the
START domain in these proteins is not known. However,
confocal fluorescent imaging demonstrated that endogenous
STARD12/DLC-1 colocalizes with caveolin-1 in BHK cells
and the two proteins co-immunoprecipitated, indicating that
STARD12/DLC-1 is localized to cholesterol and sphingo-
lipid-rich regions of the PM (Yamaga et al. 2004). The
caveolin-1 interaction is dependent on the RhoGAP domain
of STARD12/DLC-1 and the authors of this study speculated
that the START domain may bind cholesterol and regulate
STARD12/DLC-1 GAP function (Yamaga et al. 2004).
Expression of a tagged STARD13/DLC-2 in a human
hepatoma cell line was shown by confocal immunofluores-
cence imaging to be localized with mitochondria (Ng et al.
2006) and the mitochondrial association was dependent on
the START domain. Expression of the START domain of
STARD12/DLC-1 in a breast cancer cell line, on the other
hand, did not show a pattern consistent with mitochondrial
localization and the full protein was localized to the
cytoskeleton and enriched at focal adhesions (Kim et al.
2008). The ligand(s) that bind the START domain(s) of this
subfamily remain to be determined. New data on the
crystal structure for STARD13/DLC-2, however, indicate
that the STARD13/DLC-2 ligand-binding pocket is
smaller and contains polar residues that make it different
from the cholesterol and phospholipid START proteins.
The authors propose that a charged lipid would be a likely
binding candidate (Thorsell et al. 2011). Once the START
domain ligands have been defined, this information will
help to elucidate the biological significance for the START
domain within this family of RhoGAP proteins. It will be
interesting to see the effect of the lipid binding to the
START domain on RhoGAP activity and whether
subcellular localization and/or ligand binding affects
function.

STARD14/15: the acyl-CoA thioesterase subfamily.
The acyl–coenzyme A thioesterase (ACOT) family of proteins
hydrolyze the thioester bond of fatty acyl-CoAs to generate
free fatty acids and coenzyme A (reviewed in Kirkby et al.
(2010)). ACOT11_y2 and ACOT12 are unique within this
family as they contain C-terminal START domains (Hunt
et al. 2005, Kirkby et al. 2010) and are also known as
STARD14 and STARD15 respectively (SRPBCC protein
superfamily on NCBI’s Conserved Domain Database).
Human STARD14/ACOT11_y2 is a splice variant that is
the ortholog of the mouse brown fat-inducible thioesterase
(nBFIT2; Adams et al. 2001; Table 1). nBFIT2 is induced in
brown adipose tissue of mice after exposure to cold
temperatures and is expressed at higher levels in lean mouse
models compared with obese mouse models. The data suggest
an association between STARD14/ACOT11_y2/BFIT2
with increased metabolic activity in brown fat. STARD15/
ACOT12 is a cytosolic acetyl-CoA thioesterase (hydrolase)
that has been cloned from rat, mouse, and human (Suematsu
et al. 2001, 2002, Suematsu & Isohashi 2006) and is highly

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expressed in liver. The biological significance for the thioesterase activity of this enzyme should be implicit based on the fact that acetyl-CoA is the substrate, but this enzyme has been relatively understudied.

The crystal structure for the START domain of STARD14/ACOT11_v2/BFIT2 shows that the functionally critical C-terminal α helix is broken into two shorter helices (Thorsell et al. 2011). Electron density consistent with a fatty acid filled the ligand-binding cavity in the crystal, but the actual ligand and whether it is a fatty acid was not solved. It is tempting to speculate that fatty acid binding may regulate thioesterase activity of these enzymes; possibly, the START domain binds the free fatty acid product for transfer to fatty acid binding proteins, the soluble intracellular carriers of fatty acids.

Summary

One-third of the mammalian START domain proteins belong to the STARD1/D3 and STARD4 subfamilies and function to bind and transport cholesterol and oxysterols. While the biological function of STARD1 is established as the regulator of cholesterol transport across mitochondrial membranes for steroid hormone synthesis, the challenge remains to define the functions for the remaining members of the cholesterol-binding proteins. Current data suggest that both STARD4 and STARD5 associate with ER cholesterol yet serve unique roles at the ER membrane. The knockout mouse models that do not present with an apparent phenotype will require further study to determine whether exposure to environmental, dietary, or disease stressors may highlight a phenotype associated with loss of the START protein(s). Alternatively, a phenotype may only manifest upon aberrant overexpression of a START protein, as indicated by the studies that demonstrate overexpression of START proteins leads to disorders in cholesterol homeostasis in hepatocytes and macrophages. Therefore, it will be important to continue to identify START protein expression associated with different disease states that involve dyslipidemia, inflammation, and ER stress to help establish the biological significance of the in vitro data and to help distinguish unique functions from redundant functions for the STARD4 subfamily.

The phospholipid/sphingolipid-binding proteins of the STARD2/PCTP subfamily appear to have diverse functions, from modulating insulin sensitivity in liver to intermembrane transfer of ceramide, and tumor proliferation. Although the ligands are known for this subfamily, it is not yet clear what the significance is for phosphatidylcholine binding. Similarly, the next question to address for the RhoGAP and thioesterase START protein subfamilies is whether lipid binding within the START domain affects the function of the protein. The first step, however, is to determine the ligands that bind to the START domains of members of these two subfamilies.

Declaration of interest

The author declares that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review.

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Mammalian START protein functions


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