Give lipids a START: the StAR-related lipid transfer (START) domain in mammals

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Summary

The steroidogenic acute regulatory protein (StAR)-related lipid transfer (START) domain is a protein module of ~210 residues that binds lipids, including sterols. Fifteen mammalian proteins, STARD1-STARD15, possess a START domain and these can be grouped into six subfamilies. Cholesterol, 25-hydroxycholesterol, phosphatidylcholine, phosphatidylethanolamine and ceramides are ligands for STARD1/STARD3/STARD5, STARD5, STARD2/STARD10, STARD10 and STARD11, respectively. The lipids or sterols bound by the remaining 9 START proteins are unknown. Recent studies show that the C-terminal end of the domain plays a fundamental role, forming a lid over a deep lipid-binding pocket that shields the ligand from the external environment. The START domain can be regarded as a lipid-exchange and/or a lipid-sensing domain. Mammalian START proteins have diverse expression patterns and can be found free in the cytoplasm, attached to membranes or in the nucleus. They appear to function in a variety of distinct physiological processes, such as lipid transfer between intracellular compartments, lipid metabolism and modulation of signaling events. Mutation or misexpression of START proteins is linked to pathological processes, including genetic disorders, autoimmune disease and cancer.

Key words: START, Cholesterol, Phosphatidylcholine, Lipid

Introduction

The steroidogenic acute regulatory protein (StAR)-related lipid transfer (START) domain is a protein domain spanning ~210 residues (Ponting and Aravind, 1999). It is conserved through evolution in plants and animals and serves as a versatile binding interface for lipids that function in many distinct processes (Soccio and Breslow, 2003; Schrick et al., 2004). This domain is absent from yeast and Archaea but found in some protists and bacteria. In plants, the START domain is more common than in animals and is often found in homeodomain transcription factors (Schrick et al., 2004). Flies have four START proteins, which are related to mammalian STARD2/PCTP, STARD11/CERT, STARD12/DLC-1 and STARD3/MLN64. Nematodes have six, which are related to the latter four mammalian members plus STARD1/StAR and STARD10 (Soccio and Breslow, 2003).

In humans, START domains are found in 15 distinct proteins, either alone (in seven members of this family) or associated with other protein domains (in the remaining eight members) (Soccio and Breslow, 2003). The crystal structures of three of these have been solved, revealing a conserved 'helix-grip' fold that forms an inner tunnel wide enough to accommodate the hydrophobic lipid (Roderick et al., 2002; Tsujishita and Hurley, 2000; Romanowski et al., 2002). The identity of the lipids that bind each START domain is known for only a few members of the family, however. Recent work has implicated START proteins in the control of several aspects of lipid biology, including lipid trafficking, lipid metabolism and cell signaling. Moreover genetic, structural and functional studies are providing insight into the underlying mechanisms involved, as well as the distinct physiological and pathological roles of different START-domain-containing proteins. In this Commentary, we discuss these advances and the different models for START action that have been proposed. The evolution of the START domain has been discussed elsewhere (Soccio and Breslow, 2003; Schrick et al., 2004). We therefore focus here on the mammalian START proteins.

Six START subfamilies in mammals

The cloning of both the steroidogenic acute regulatory protein (StAR, also known as STARD1), a mitochondrial cholesterol carrier synthesized following trophic hormone stimulation of MA-10 Leydig tumor cells (Clark et al., 1994), and metastatic lymph node 64 (MLN64, also known as STARD3), a protein overexpressed in breast cancer, revealed that they share a conserved domain at their C-termini (Moog-Lutz et al., 1997; Watari et al., 1997). This ~210-residue conserved region was subsequently found to be present in several proteins and designated the START domain (Ponting and Aravind, 1999). A multiple sequence alignment of the 15 START domains using ClustalW (Thompson et al., 1994) in humans allows construction of a phylogenetic tree that divides the family into six subfamilies (Fig. 1).

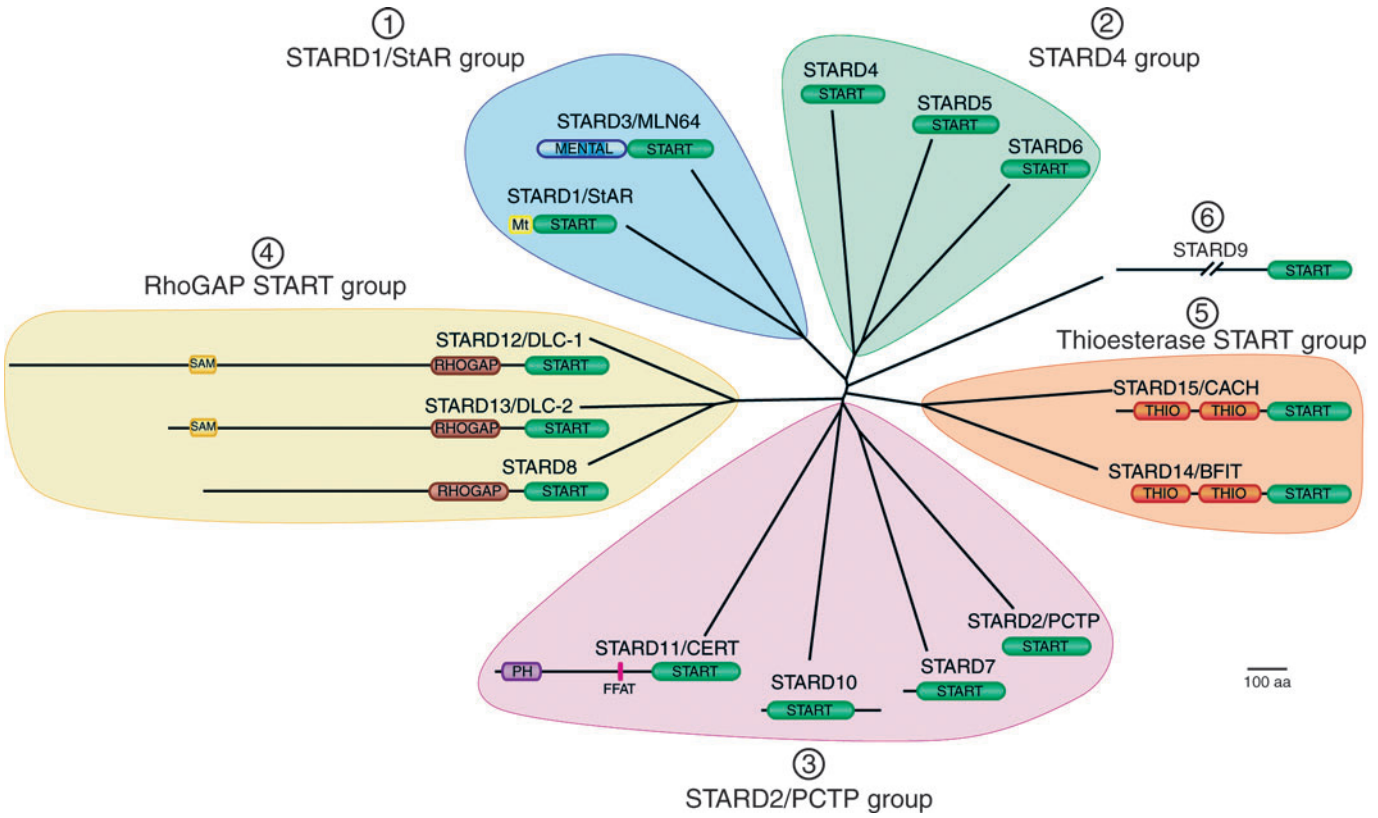

Fig. 1. Phylogenetic tree and domain organizations of the 15 START-domain proteins in humans. START domain sequences were aligned by the Eclustalw program (Genetics Computer Group, Madison, WI). The phylogenetic tree was drawn with the drawtree software [J. Felsenstein, 1993, PHYLIP (Phylogeny Inference Package) v.3.5c, Department of Genome Sciences, University of Washington, Seattle, WA]. Abbreviations: Mt, mitochondrial targeting motif; MENTAL, MLN64 N-terminal domain; PH, pleckstrin homology domain; FFAT, two phenylalanines in an acidic tract motif responsible for ER targeting; RHOGAP, Rho-GTPase-activating-protein domain; SAM, sterile alpha motif; THIO, acyl-CoA thioesterase domain.

The STARD1/StAR group of intracellular cholesterol carriers

STARD1/StAR

StAR regulates the limiting step in steroid hormone production in response to hormonal stimuli by mobilizing cholesterol at mitochondrial membranes (Clark et al., 1994; Clark and Stocco, 1995; Stocco, 2001). Cholesterol is initially present in the outer mitochondrial membrane, StAR appears to be essential for its transfer to the inner mitochondrial membrane (Clark and Stocco, 1995; Stocco, 2001). In the inner mitochondrial membrane, the cholesterol side chain is then cleaved by the P450 side chain cleavage complex (P450scc), generating pregnenolone. In steroidogenic cells, *StAR* expression is tightly regulated (Manna et al., 2003; Stocco et al., 2005). Forced expression of StAR in MA-10 cells, which express P450scc, can induce the conversion of cholesterol into pregnenolone in the absence of hormonal stimulation (Sugawara et al., 1995; Lin et al., 1995). The critical role of StAR in steroidogenesis is evident from analysis of individuals with lipoid congenital adrenal hyperplasia (CAH) (Lin et al., 1995). Lipoid CAH is characterized by a pathological accumulation of cholesterol in the gonads and adrenals along with steroid synthesis deficiency, and some patients possess mutations in the *StAR* gene (Lin et al., 1995; Stocco, 2002). Disruption of the *StAR* gene in mice results in a similar disorder (Caron et al., 1997).

STARD3/MLN64

MLN64, like StAR, is a cholesterol-specific START protein. *MLN64* was identified as a gene overexpressed in malignant compared with benign breast tumours (Tomasetto et al., 1995). It is overexpressed in about 25% of breast cancers (Bieche et al., 1996 ; Kauraniemi et al., 2001 ; Pollack et al., 2002; Hyman et al., 2002; Dressman et al., 2003). MLN64 and StAR are differentially localized in cells (Fig. 3) (Clark et al., 1994; Alpy et al., 2001) but have similar biophysical and functional properties (Tuckey et al., 2004). It may therefore be their expression patterns and subcellular localizations that distinguish them. The function of MLN64 remains elusive. The full-length protein has negligible steroidogenic activity, but a mutant containing only the START domain significantly promotes steroidogenesis by P450scc (Watari et al., 1997) and can bind cholesterol at a 1:1 ratio (Tsujishita and Hurley, 2000). MLN64 could thus function in steroidogenesis in organs that do not express StAR, such as the placenta (Watari et al., 1997). However, mice lacking the MLN64 START domain appear normal and show no defect in steroidogenesis, making this unlikely (Kishida et al., 2004).

Given the presence of the START domain and its position at the limiting endosomal-membrane, MLN64 was proposed to function in cholesterol homeostasis by mediating mobilization of endosomal cholesterol to a cytosolic acceptor or membrane (Alpy et al., 2001). Interestingly, MLN64 contains a conserved N-terminal region, the MENTAL domain, which it shares with a unique protein, MENTHO, and anchors it to endosome membranes, leaving the C-terminal START domain in the cytoplasm (Alpy et al., 2002). This domain is both a protein-protein interaction and a cholesterol-binding module. It mediates homo and hetero-interaction of MLN64 and MENTHO and binds photocholesterol in vivo (Alpy et al., 2005). The later finding supports the proposed mode of MLN64 action in cholesterol transport. MLN64 could capture cholesterol by its MENTAL domain in the late endosome membranes; this could then be extracted by its cytoplasmic START domain and transferred to a cytosolic acceptor.

The STARD4 group

The related START-only proteins, STARD4, STARD5 and STARD6 were recently isolated by genomic studies after identification of STARD4 as an expressed sequence tag downregulated in mice maintained on a high-cholesterol diet (Soccio et al., 2002). Forced expression of STARD4 or STARD5 stimulates steroidogenesis by P450scc and liver X receptor reporter gene activity, thus indicating that both proteins function in cholesterol metabolism and might be cholesterol or sterol-specific binding proteins (Soccio et al., 2005). Indeed, STARD5 was recently found to bind cholesterol and 25-hydroxycholesterol and no other sterols (Rodriguez-Agudo et al., 2005). The lipid specificity of STARD6 is not known. *STARD4* is induced by sterol-regulatory binding proteins and *STARD5* expression is increased by endoplasmic reticulum stress (Soccio et al., 2005). STARD5 is upregulated in lung cancers (Table 1). Although expressed in the same tissues, the *STARD4* and *STARD5* genes are differentially regulated , which suggests that they have distinct functions in cholesterol metabolism (Soccio et al., 2002; Soccio et al., 2005). By contrast, STARD6 is restricted to the testes and is expressed during spermatogenesis in spermatids but not in steroidogenic cells (Soccio and Breslow, 2003; Gomes et al., 2004). Because sterols and lipids play an important role in sperm function, and the plasma membrane cholesterol/phospholipid ratio falls during sperm capacitation (Travis and Kopf, 2002), STARD6 might regulate lipid movement within the sperm cell membrane. Interestingly, STARD6 has been detected in the nucleus of mature rat sperm

Table 1. Human START-domain proteins

Name	Other names[a]	Lipid specificity[b]	Subcellular localization[c]	Expression pattern[d]	Mainly expressed[e]	Implicated in[f]	Gene chromosomal localization[g]
STARD1	StAR	Cholesterol[1]	Mitochondria[1]	Specific	Adrenal, Gonads, Brain[1]	Genetic disorder[1]	8p11.2
STARD2	PCTP	Phosphatidylcholine[2]	Cytosol[2]	Wide			17q21-q24
STARD3	MLN64, CAB1	Cholesterol[1]	Late-endosomes[3]	Wide		↑ Cancer[2]	17q11-q12
STARD4	None		Cytosol and Nucleus[4]	Wide			5q22.1
STARD5	None	Cholesterol, 25-hydroxycholesterol[3]	Cytosol and Nucleus[5]	Wide		↑ Cancer[3]*	15q26
STARD6	None		Nucleus?[6]	Specific	Testis[2]		18q21.2
STARD7	GTT1		?	Wide		↑ Cancer[4]	2q11.2
STARD8	RhoGAP		?	Wide	Placenta, PNS	↓ Cancer[5]*	Xq13.1
STARD9	None		?	Wide		↓ Cancer[6]*	15q15.1-q15.2
STARD10	PTCP-like, SDCCAG28, CGI-52	Phosphatidylcholine/ethanolamine[4]	Cytosol and Nucleus[7]	Wide		↑ Cancer[7]	11q13
STARD11	CERT, GPBP, COL4A3BP	Ceramides[5]	Cytosol and Golgi[8]	Wide		Autoimmune disease[8]	5q13.3
STARD12	DLC-1,Arhgap7, p122-RhoGAP		Plasma membrane[9]	Wide		↓ Cancer[9]	8p22
STARD13	DLC-2, SDCCAG13		Cytosol?[10]	Wide		↓ Cancer[10]	13q12-q13
STARD14	BFIT, THEA		?	Wide		Obesity[11]	1p32.3
STARD15	CACH		Cytosol[11]	Specific	Liver, Lung	↓ Cancer[12]*	5q14.1

The same color field was used for the members of a given subfamily as defined in Fig. 1.

Abbreviations: BFIT, brown fat-inducible thioesterase; CAB1, coamplified with erbB2 1; CACH, cytoplasmic acetyl-CoA hydrolase; COL4A3BP, collagen type IV alpha 3 binding protein; DLC-1, deleted in liver cancer 1; GPBP, Goodpasture-antigen-binding protein; GTT1, gestational trophoblastic tumor 1; MLN64, metastatic lymph node 64; PCTP, phosphatidylcholine tranfer protein; SDCCAG, serologically defined colon cancer antigen; StAR, steroidogenic acute regulatory protein; THEA, thioesterase-adipose-associated protein.

[b]Lipid specificity according to literature: [1]Tsujishita and Hurley, 2000; [2]Wirtz, 1991; [3]Rodriguez-Agudo et al., 2005; [4]Olayioye et al., 2005; [5]Hanada et al., 2003.

[c]Localization according to literature: [1]Clark et al., 1994; [2]de Brower et al., 2002; [3]Alpy et al., 2001; [4]Soccio et al., 2005 and Alpy et al., 2005; [5]Soccio et al., 2005; [6]Gomes et al., 2004; [7]Yamanaka et al., 2000 and Olayioye et al., 2004; [8]Hanada et al., 2003; [9]Yamaga et al., 2004; [10]Ching et al., 2003; [11]Suematsu et al., 2001; ?, unknown or needs further confirmation.

[d]Expression profile suggested by counting expressed sequence tags (ESTs) from normal human tissues using UniGeneEST Profile viewer (NCBI). EST libraries from bladder, blood, bone marrow, brain, cervix, colon, eye, heart, kidney, larynx, liver, lung, lymph node, mammary gland, muscle, ovary, pancreas, peripheral nervous system, placenta, prostate, skin, small intestine, soft tissue, stomach, tongue, testis, thymus, uterus and vascular tissue were included. Expression was considered to be tissue specific (Specific) when ESTs were found in 10 or less distinct tissues. When ESTs were found in more than 10 distinct organs, genes were considered to be widely expressed (Wide).

[e]Expression according to literature: [1]Stocco, 2001 and King et al., 2004; [2]Soccio et al., 2002 and Gomes et al., 2004. For STARD8 and STARD15, results were suggested from UniGeneEST Profile viewer.

[f]According to literature or *to the cancer microarray database Oncomine (http://www.oncomine.org) (Rhodes et al., 2004a; Rhodes et al., 2004b), ↑ or ↓ signify upregulated or downregulated in cancer versus normal samples, respectively: [1]Stocco, 2002; [2]Tomasetto et al., 1995; [3]Bhattacharjee et al., 2001; [4]Durand et al., 2004; [5]LaTulippe et al., 2002 and Singh et al., 2002; [6]Garber et al., 2001; [7]Olayioye et al., 2004; [8]Raya et al., 1999; [9]Yuan et al., 1998; [10]Ching et al., 2003; [11]Adams et al., 2001; [12]Chen et al., 2002.

[g]According to Unigene mapping position (NCBI).

cells (Gomes et al., 2004), where it could interact with transcriptional machinery in a lipid-dependent manner.

The STARD2/PCTP group of lipid transporters of lipids

Phylogenetic analysis groups together phosphatidylcholine transfer protein, (PTCP, also known as STARD2), STARD7, STARD10 and STARD11 [also known as Goodpasture-antigen-binding protein (GPBP) or CERT]. This group is more heterogeneous than the others since the genes do not share common exonic organization and two of the proteins bind different lipids.

STARD2/PCTP

PCTP is a cytosolic lipid-specific transfer protein that promotes the rapid exchange of phosphatidycholine (PC) between membranes (Wirtz, 1991). PCTP-deficient mice appear normal (van Helvoort et al., 1999), and the biological function of PCTP remains ill defined. It is believed to shuttle PC from its site of synthesis in the ER to the inner layer of the plasma membrane and/or the outer membrane of the mitochondria. This is thought to replenish plasma membrane with PC in response to phospholipid efflux during high-density lipoprotein (HDL) transport between tissues (Baez et al., 2002; Baez et al., 2005). Photobleaching experiments showed that PCTP is very mobile in the cytoplasm (de Brouwer et al., 2002). Interestingly, in response to clofibrate treatment (a PPARα agonist), PCTP becomes associated with mitochondria (de Brouwer et al., 2002). This recruitment is associated with phosphorylation of the protein on serine 110, a conserved residue that is also phosphorylated in StAR (de Brouwer et al., 2002). The precise role of this relocalization is unclear but suggests a potential mitochondrial function for PCTP (de Brouwer et al., 2002).

STARD7

STARD7, also known as gestational trophoblastic tumour 1 (GTT1), was isolated as a gene overexpressed in choriocarcinoma (Durand et al., 2004). Its broad expression pattern indicates it might have a role in phospholipid transport. However, its common upregulation in many cancer-derived cell lines means it might play a role in phospholipid-mediated tumour signaling (Durand et al., 2004). Unlike PCTP, its lipid specificity is not known.

STARD10

STARD10 (previously named PCTP-like) is widely expressed and synthesized constitutively in many organs, including liver, where it might act in export of lipids into bile. Recently, STARD10 was found to function as a phospholipid transfer protein by binding to phophatidylcholine and phosphatidylethanolamine (Oliayioye et al., 2005). STARD10 expression is also regulated during development in the testes and mammary glands (Yamanaka et al., 2000). The protein is concentrated in the sperm flagellum. Because enzymes involved in energy production are located in flagella and PC could be a potential substrate for this, STARD10 might play a role in energy metabolism by mobilizing PC (Yamanaka et al., 2000). Interestingly, STARD10 and STARD6 show similar expression patterns in testes and may thus be partners in sperm cells. STARD10 expression is induced in mammary gland during gestation and lactation (Olayioye et al., 2004). It is also upregulated in tumors from the mammary glands of transgenic mice expressing activated ErbB-2, a member of the epidermal growth factor (EGF) receptor family, and overexpressed in tumor-derived cell lines and 50% of ErbB-2-positive breast tumors (Olayioye et al., 2004). The relationship between STARD10 and EGF receptors is unclear. However, in cotransfected NIH3T3 cell lines, STARD10 cooperates with ErbB-1 to promote anchorage-independent growth (Olayioye et al., 2004).

PTCP, STARD7 and STARD10 are co-expressed in the liver, where they could function in the secretion of lipids into the bile. The absence of PTCP in mice does not impair PC secretion into bile (van Helvoort et al., 1999) possibly because this function is rescued by STARD7 and/or STARD10.

STARD11/CERT

STARD11, the only remaining START protein whose lipid specificity is known, is synthesized from two main transcripts: a long one encoding Goodpasture-antigen-binding protein (GPBP), also named CERTL; and a shorter one lacking one exon, GPBPΔ26 (also known as CERT) (Raya et al., 2000; Hanada et al., 2003). The shorter transcript is the more abundant. In humans, STARD11 is expressed in many tissues, including skeletal muscle, heart, brain, kidney, pancreas and placenta (Raya et al., 2000). STARD11 is composed of an N-terminal pleckstrin homology (PH) domain, a serine-rich motif, a potential coiled-coil region, a FFAT (two phenylalanine amino acids in an acidic tract) motif, a second 26-residue serine-rich motif (deleted in GPBPΔ26/CERT) and a C-terminal START domain. Recombinant STARD11 binds and phosphorylates Goodpasture antigen, the C-terminal region of the α3 chain of collagen IV, which is involved in the autoimmune disease Goodpasture disease (Raya et al., 1999). The role of STARD11 in Goodpasture disease is unclear; however, it is expressed in cells and tissues targeted by the autoimmune response. STARD11 might phosphorylate Goodpasture antigen and trigger its processing and peptide presentation and thus mediate autoimmunity (Raya et al., 1999; Raya et al., 2000).

STARD11 was recently shown to act as a non-vesicular ceramide-carrier protein (Hanada et al., 2003). Ceramide is the precursor of sphingolipids, an abundant component of cell membranes. Ceramides are synthesized in the ER. They reach the Golgi apparatus by a major non-vesicular, ATP-dependent route and are then converted into sphingolipids. STARD11 rescues a mutant cell line that cannot transport ceramide from the ER to the Golgi (Hanada et al., 1998; Hanada et al., 2003). Distinct protein domains within the protein cooperate (Hanada et al., 2003). The recently described FFAT motif binds to an ER membrane protein called vesicle-associated membrane-protein-associated protein (VAP) (Loewen et al., 2003) and the PH domain targets STARD11 to the Golgi by interacting with phosphatidylinositol-4 monophosphate (Levine and Munro, 2002). Deletion mutants reveal that only the START domain mediates ceramide transfer and is responsible for the specific exchange of ceramide from donor to acceptor membranes (Hanada et al., 2003). STARD11 can efficiently transfer several

natural ceramide species possessing long saturated acyl chains (C14-C20), C16-dihydroceramide and C16-phytoceramide (Kumagai et al., 2004).

The RhoGAP START group

The RhoGAP START subfamily comprises deleted in liver cancer 1 (DLC-1, also known as STARD12 or p122), deleted in liver cancer 2 (DLC-2, also known as STARD13) and STARD8. Each has a Rho GTPase-activating protein (RhoGAP) domain and a C-terminal START domain. DLC-1 and DLC-2 each also possess an N-terminal sterile alpha motif (SAM) domain. The SAM domain is present in proteins involved in many biological processes and seems to have a variety of functions (Kim and Bowie, 2003), such as homo- and hetero-oligomerization, RNA binding and lipid binding (Barrera et al., 2003). RhoGAP domains regulate the activity of Rho-family small GTPases by stimulating their inherent GTPase activity (Moon and Zheng, 2003).

STARD12/DLC-1

The DLC-1 gene is a potential tumor suppressor gene located on chromosome 8 p21-22, a region of frequent loss of heterozygosity in human cancers (Yuan et al., 1998). It is deleted in liver and breast primary tumors (Yuan et al., 1998; Yuan et al., 2003b; Wong et al., 2003) and downregulated in human liver, breast, colon and prostate cancer cell lines (Yuan et al., 2003a; Plaumann et al., 2003). Expression of DLC-1 in cell lines derived from liver, lung and breast carcinomas inhibits cell growth, colony formation and tumorigenicity in nude mice (Ng et al., 2000; Yuan et al., 2003b; Yuan et al., 2004; Zhou et al., 2004; Plaumann et al., 2003). DLC-1 is a bi-functional protein. First, it interacts with PLC-δ1 in vivo and stimulates hydrolysis of phosphatidylinositol 4,5-bisphosphate [PtdIns (4,5)P_2], generating inositol 1,4,5-triphosphate and thus Ca^{2+} release from intracellular stores (Homma and Emori, 1995). This activity depends on the C-terminal half of DLC-1, which encompasses the GAP and START domains (Sekimata et al., 1999). Second, DLC-1 stimulates the intrinsic GTPase activity of RhoA, but not of Rac1, K-Ras, Rab3 or Cdc42Hs (Homma and Emori, 1995). Expression of DLC-1 changes cell shape by inducing cell rounding and disassembly of stress fibers in a GAP-dependent manner. These morphological modifications are regulated by Rho GTPases (Sekimata et al., 1999).

Rat DLC-1 localizes to the plasma membrane in caveolae, where it interacts with caveolin-1 (Yamaga et al., 2004), and in focal adhesions where it colocalizes with vinculin at the tips of actin stress fibers (Kawai et al., 2004). The GAP domain alone is sufficient to localize DLC-1 to caveolae (Yamaga et al., 2004), whereas the N-terminal part of the protein including the SAM domain targets it to focal adhesions (Kawai et al., 2004). The DLC-1 knockout is lethal. Embryonic fibroblasts derived from DLC-1-deficient mouse embryos display alterations in the organization of actin filaments and focal adhesions, emphasizing its essential function in the cytoskeleton (Durkin et al., 2005). Indeed, DLC-1 inactivation might contribute to the changes in cytoskeletal organization commonly found in cancer cells.

STARD13/DLC-2

DLC-2 is another potential tumor suppressor gene (located on chromosome 13q12.3) (Ching et al., 2003). Indeed, loss of its chromosomal region is common in hepatocellular carcinomas (HCC) and other cancers. DLC-2 is widely expressed, and the recombinant protein has GAP activity towards RhoA, Cdc42 and, to a lesser extent, Rac1 (Ching et al., 2003; Nagaraja and Kandpal, 2004). Its GAP domain can inhibit Rho-mediated cytoskeletal reorganization and stress fiber formation, which indicates that DLC-2 acts as a RhoGAP in vivo (Ching et al., 2003; Nagaraja and Kandpal, 2004).

STARD8

STARD8 lacks the N-terminal SAM domain present in the other member of this subfamily. Again it is widely expressed and downregulated in certain cancers (Table 1).

The sequences of DLC-1, DLC-2 and STARD8 are very similar, sharing >50% identity. All three are probably involved in cytoskeletal organization. In this subfamily, the START domain could have a regulatory role that is dependent on lipid.

The thioesterase START group

Brown fat-inducible thioesterase (BFIT, also known as STARD14) and cytoplasmic acetyl-CoA hydrolase (CACH, also known as STARD15) both contain two N-terminal acyl-CoA hydrolase domains and a C-terminal START domain (Fig. 1). They are serine esterases that have an active serine residue in the catalytic site and are similar to prokaryotic acyl-CoA thioesterases. BFIT hydrolyses medium- (C12-CoA) and long-chain (C16-CoA) fatty acyl-CoA substrates (Adams et al., 2001). CACH preferentially hydrolyses acetyl-CoA (Prass et al., 1980; Suematsu et al., 2001).

STARD14/BFIT

BFIT is induced in the brown adipose tissue of cold-challenged animals and repressed in animals at warmer temperatures (Adams et al., 2001), although it is widely expressed in humans. Two splice variants have been described: BFIT1 (607 amino acids) and BFIT2 (594 amino acids), which differ at their C-termini. This difference may affect the START domain since only BFIT2 possesses the C-terminal α4 helix found in other START proteins. Significantly, mice only have the BFIT2 isoform (Adams et al., 2001).

Mouse *BFIT* is located in the genomic region containing the dietary-obese 1 (*Do1*) locus, which includes gene(s) potentially involved in body fat control. BFIT is more highly expressed in the brown adipose tissue of obesity-prone compared with obesity-resistant or lean mice (Adams et al., 2001). Interestingly, the chromosomal region containing the human *BFIT* gene is linked to body mass index and fat mass (Adams et al., 2001). Given its expression pattern and enzymatic activity, BFIT probably has an important function in lipid metabolism.

STARD15/CACH

In mice, CACH expression is restricted to certain organs,

including the liver, kidney, spleen, muscle and testes (NCBI UniGene EST Profile Viewer). This enzyme is only active as homodimers or tetramers (Isohashi et al., 1983b) and is allosterically activated by ATP and inhibited by ADP (Isohashi et al., 1983a). Strikingly, CACH activity increases in opposing metabolic states, such as fatty acid synthesis and degradation (β-oxidation) (Matsunaga et al., 1985). Moreover, CACH has been linked to cholesterol metabolism, because its activity increases when cholesterol synthesis is inhibited (by chemical agents) or reduced (by high-cholesterol diets) (Ebisuno et al., 1988). Given its preference for acetyl-CoA, CACH probably acts to maintain the equilibrium between cytoplasmic acetyl-CoA and CoA-SH available for fatty acids and cholesterol metabolism.

STARD9

Very little is known about STARD9. It is predicted to encode a large, >1820 residues protein containing a C-terminal START motif (Fig. 1). Besides the START domain, no other known region has been identified within its open reading frame.

The 3D structure of the START domain

The crystal structures of the START domains of MLN64, PCTP and STARD4 have been solved (Tsujishita and Hurley, 2000; Roderick et al., 2002; Romanowski et al., 2002). All three show similar structural features (Fig. 2). They adopt a 'helix-grip' fold, in which a central antiparallel β-sheet is gripped by N-terminal and C-terminal α-helices (α1 and α4), the latter being closely packed above the nine-strand curved β-sheet (Iyer et al., 2001). Two Ω loops are inserted between strands β5 and β6 (Ω1) and strands β7 and β8 (Ω2). The 3D organization of the domain forms an inner tunnel (Fig. 2). The curved β-sheet, three α-helices (α2, α3, α4) and a loop (Ω1) form the walls of a hydrophobic tunnel that is wide enough to accommodate one molecule of cholesterol in MLN64 (Fig. 2). Moreover, PCTP has been crystallized with one molecule of its PC ligand inside the tunnel. The tunnel possesses two narrow openings, which are too small to allow the entrance or exit of the ligand without major structural rearrangement of the domain. Such reorganization could involve the C-terminal α4 helix, which acts as a lid over the curved β-sheet, and maybe the loop Ω1. Indeed α4 must play a crucial role in the biological function of the START domains of PCTP and StAR, because C-terminal truncations of 10 or 28 amino acids, respectively, abolish their activity (Feng et al., 2000; Arakane et al., 1996).

Strikingly, the 3D structures of two plant lipid-binding proteins, Bet v 1 and Pru av 1 (Gajhede et al., 1996; Neudecker et al., 2001), are very similar to that of the START domain, although they do not share any sequence identity with it. Similar folds may therefore be present in other lipid-binding proteins.

Ligand specificity

Cholesterol, 25-hydroxycholesterol, phosphatidylcholine, phosphatidylethanolamine and ceramides are the ligands for StAR/MLN64/STARD5, STARD5, PTCP/STARD10, STARD10 and STARD11, respectively. The lipids bound by the remaining START proteins are unknown. Tsujishita and Hurley (Tsujishita and Hurley, 2000) have examined the determinants of ligand specificity by structure-based alignment of representative START domain sequences, using conserved and modified residues lining the tunnel wall to identify residues likely to be involved in ligand interaction (Tsujishita and Hurley, 2000). For example, at the same spatial localization, the two cholesterol-binding residues Met307 and Asn311 in MLN64 are conserved in StAR but not in PCTP. Instead, two charged residues, Arg78 and Asp82, occupy these positions: Arg78 interacts directly with the phosphoryl group of PC and

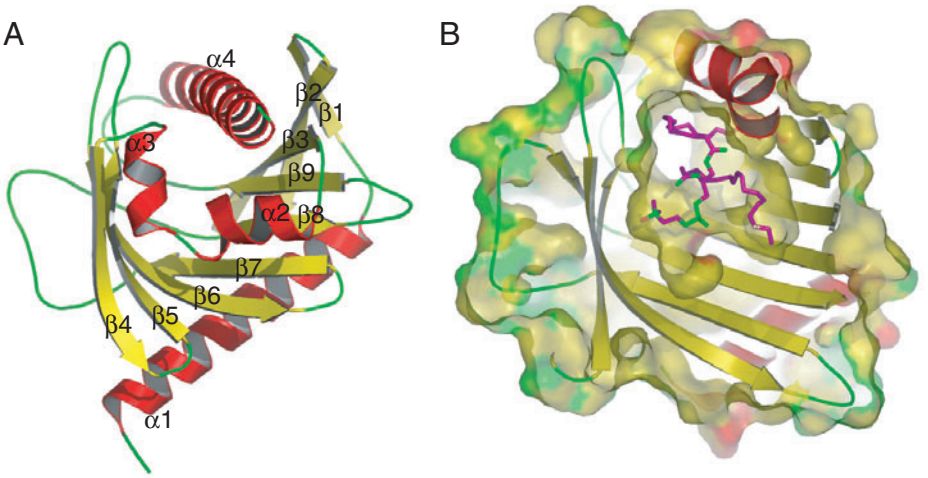

Fig. 2. Structure of the START domains of MLN64 (A) and PCTP with its ligand (B). (A) Ribbon diagram of the START domain of MLN64 (PDB ID code: 1EM2). Secondary structural elements and the C- and N-termini are labeled. MLN64 has a central β-sheet (yellow) gripped by N-terminal (α1) and C-terminal (α4) α-helices (red), the latter being closely packed above the curved sheet. (B). Cut-away view of the molecular surface of the START domain of PCTP complexed with a phosphatidylcholine molecule (DLPC, dilinoleoyl-*sn*-glycerol-3-phosphorylcholine) (PDB ID code: 1LN1). The DLPC molecule (shown in stick representation) is located in the hydrophobic tunnel formed in the START domain. The orientation of the START domain is similar to that in A. The protein surface is colored according to secondary structure: red for α helices, yellow for β strands and green for loops. These figures were prepared with PyMOL software (W. L. DeLano, 2002, The PyMOL molecular graphics system. http://www.pymol.org).

forms a salt bridge with Asp82 (Roderick et al., 2002). These two residues contribute to the specificity of the START domain for hydrophobic or charged lipid ligands. Accordingly, mutation of them to arginine and aspartate in MLN64 completely abolishes cholesterol binding (F.A., unpublished). Interestingly, examination of the sequences of all the mammalian START-containing proteins that have no known ligand suggests that charged lipids might preferentially bind to STARD4, STARD5, STARD6, STARD7, STARD8, STARD13 (DLC-2), STARD14 (BFIT) and STARD15 (CACH).

Lipid sensing

In some START proteins, the START domain probably simply functions in lipid sensing rather than in lipid transfer (see below). For instance, START domains are common in homeodomain (HD) transcription factors in plants (Schrick et al., 2004) and their lipid ligands might thus modulate transcription. No HD-START protein has been found in mammals, although a recent study noted the nuclear localization of STARD6 in male germ cells (Gomes et al., 2004). Interestingly, PCTP shows a cytoplasmic and nuclear localization (de Brouwer et al., 2002) and EGFP-STARD4 gives a nucleo-cytoplasmic signal when transiently expressed in HeLa cells (Fig. 3). In addition, STARD10 is detected in the cytoplasm and nucleus in breast cancer cells (Olayioye et al., 2004). It is therefore possible that, in mammals, some START-containing proteins have nuclear roles and may even regulate transcription in a lipid-dependent manner.

The function of RhoGAP START proteins might be regulated by the START domain in a lipid-dependent manner. Structurally, the RhoGAP START proteins resemble chimaerin proteins, which contain a RacGAP domain and a lipid-binding domain specific for diacylglycerol/phorbol-ester, the C1 domain (Brose and Rosenmund, 2002). Binding of phospholipids causes chimaerins to translocate to the Golgi apparatus and plasma membrane, and alters the conformation of the protein, allowing activation of the GAP domain (Canagarajah et al., 2004). The RhoGAP START proteins might operate similarly, modulating the activity of RhoGAP and SAM domains in a lipid-dependent manner.

Similarly, within the thioesterase START group, the START domain could function as a lipid-sensing domain, providing a rapid way of regulating the catalytic activity of BFIT and CACH, and thus modulate lipid metabolism.

Mechanism of lipid exchange

Aside from lipid binding, a common function of several START proteins appears to be exchange of lipids between membranes. The dynamics of lipid exchange mediated by START proteins are not well understood. However, we can put forward a potential model of action based on the extensive studies of StAR. StAR has a mitochondrial targeting sequence at its N-terminus that is removed upon import into the mitochondrial matrix, where the mature StAR protein is ultimately degraded (Clark and Stocco, 1995; Stocco, 2001). Several models have been proposed to explain StAR-mediated cholesterol transfer: the first proposed that StAR acts during its import into this organelle (Stocco and Clark, 1996); the second invokes action at the outer mitochondrial membrane prior to its import (Bose et al., 2000); and the third postulates a cholesterol-binding shuttling protein able to transfer cholesterol across the intermembrane space (Tsujishita and Hurley, 2000).

Surprisingly, impairing targeting of StAR to mitochondria by removing the 62 N-terminal residues has no effect on steroidogenesis monitored in vitro (Arakane et al., 1996). Moreover, StAR is inactive when trapped in the inner mitochondrial matrix or at the inner mitochondrial membrane (Bose et al., 2002). In addition, biophysical studies of the N-terminally deleted forms of StAR have determined that StAR has a molten globule structure at low pH in solution and in association with membranes, and that the transition to this state is associated with cholesterol release (Bose et al., 2000; Christensen et al., 2001). Other analyses have shown that the α4 helix of the START domain of StAR can bind to synthetic membranes in a pH-dependent manner (Yaworsky et al., 2005). Together, these data support the second model, indicating that StAR acts at the surface of the outer mitochondrial membrane. Upon interaction with the outer mitochondrial membrane, contact between the α4 helix of the START domain and acidic phospholipid heads might change the conformation of the protein and open the α4 'lid' to allow delivery (Fig. 4). Import of StAR into the mitochondrial matrix thus appears secondary to its action in steroidogenesis and instead probably terminates steroidogenesis (Bose et al., 2002; Granot et al., 2003).

We can extrapolate this model to accommodate other START proteins. Indeed, Feng et al. have shown that the C-terminal region of PCTP including the α4 helix has a role in membrane binding and facilitates PC extraction (Feng et al., 2000). Fig. 4 illustrates how different START proteins might act in lipid transfer between membranes. For START only

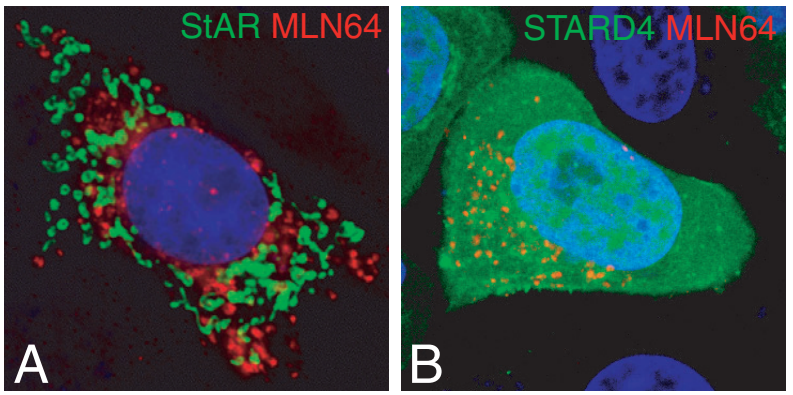

Fig. 3. START-containing proteins have distinct subcellular localization. (A) Cotransfection of HeLa cells with StAR (green) and MLN64 (red). MLN64 and StAR show a vesicular staining pattern corresponding to endosomes and mitochondria, respectively. (B) Cotransfection of HeLa cells with MLN64 (red) and GFP-STARD4 (green). MLN64 shows a typical punctate staining corresponding to endosomes whereas the GFP-STARD4 fusion protein gives a diffuse nuclear and cytoplasmic signal. Nuclei were counterstained with Hoechst-33258 dye.

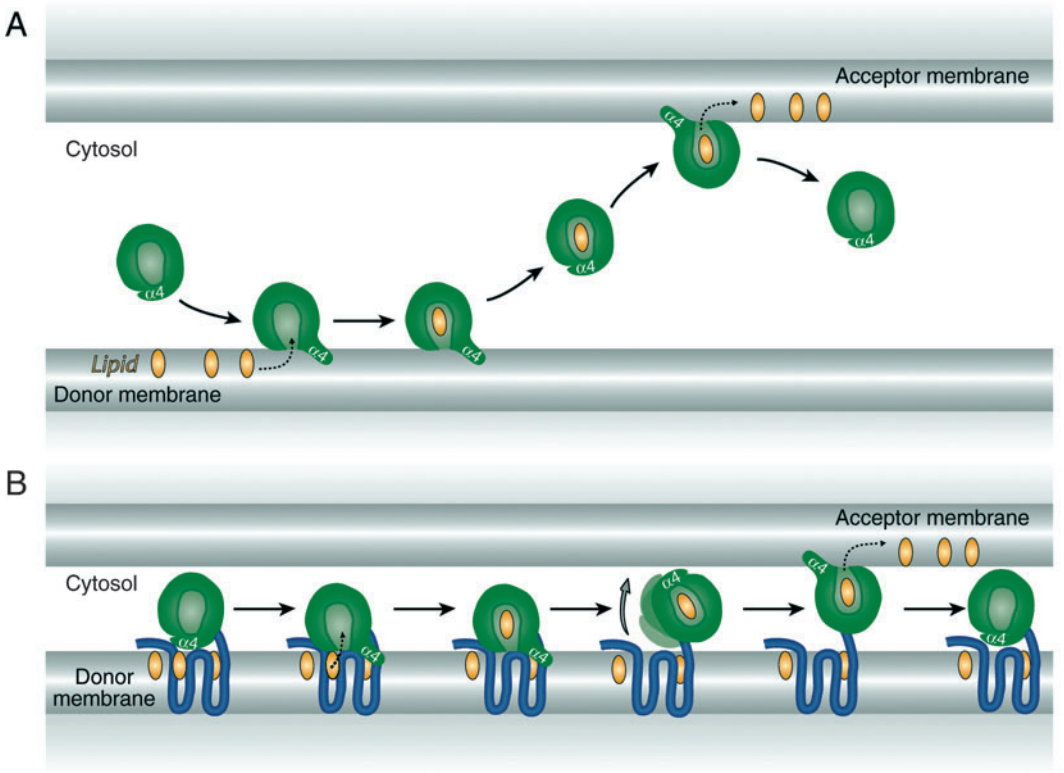

Fig. 4. Conveying lipids across the cytoplasm. (A) Mode of action of a START-only protein. In its unliganded form, the START domain interacts with the membrane through its C-terminal α4 helix. This interaction induces a conformational change and opens the lipid-binding pocket. When the lipid (yellow) is occupying the pocket, the protein conformation changes again and the lid is closed. The liganded form of the START protein must be targeted to an acceptor membrane to deliver its lipid. (B) Mode of action of MLN64. The MENTAL domain of MLN64 (blue) anchors it to endosome membranes, leaving its C-terminal START domain (green) in the cytoplasm. The START domain of MLN64 might work by extracting cholesterol (yellow) bound to its MENTAL domain from the late endosome membrane and transfer it to a closely positioned acceptor membrane. This model would allow significant and rapid cholesterol transfer.

proteins, including STARD4, STARD5, STARD6, PTCP, STARD7 and STARD10 (Fig. 4A), the α4 helix opens the lipid-binding pocket upon interaction with the membrane. After lipid absorption, the pocket is closed and the START protein can then exchange its lipid with another membrane. The mechanisms responsible for targeting START proteins to specific donor or acceptor membranes are not clear. Post-translational modifications such as phosphorylation and/or interaction with other proteins may well govern this. It is tempting to speculate that the conformation of the protein favors recruitment to a specific membrane upon ligand binding, whereas the protein moves back to a different site after lipid desorption. In the case of START proteins containing membrane-targeting motifs, such as StAR, STARD11 and MLN64, we can speculate that these proteins localize to specific contact sites through targeting by other domains and/or interaction with specific membrane-resident proteins.

Intracellular contact sites between different membranes have been seen by microscopy (Holthuis and Levine, 2005). STARD11, using its FFAT and PH domains at the same time, could draw together components of the ER and Golgi. In this scenario, the START domain could extract and deliver ceramide by a flipping mechanism (Munro, 2003). Similarly, in transfected cells, MLN64-containing late endosomal tubules align parallel to StAR-labeled mitochondria and transiently contact these (Zhang et al., 2002). As shown in Fig. 4B, the MENTAL domain of MLN64 anchors the protein at the periphery of late endosomes, it also may capture cholesterol within the late endosome membranes, and the cytoplasmic START domain could extract cholesterol prior to its transfer to an acceptor membrane (Fig. 4B).

The recruitment of START proteins to specific contact sites would reconcile two contrasting observations about the function of the START domain: it binds only 1 mole of ligand per mole of protein but must handle several ligand molecules in a very short time. Indeed, StAR transfers over 400 molecules of cholesterol/StAR/minute (Artemenko et al., 2001). If acceptor and donor sites are brought together, one START protein could mediate such a rapid and efficient exchange of many ligand molecules.

To date, only membranes have been identified as lipid-exchange partners for START proteins. However START proteins might exchange lipids with acceptor proteins.

Conclusions and perspectives

The START domain acts as a shield to protect a hydrophobic lipid from a hydrophilic environment. It operates as a lipid-exchange and/or a lipid-sensing domain. START proteins are involved in several different biological processes: lipid transfer

between cellular compartments; lipid metabolism, which involves START proteins that contain thioesterase catalytic activities; and signal transduction, which involves the RhoGAP START proteins. Within the START domain, the C-terminal α helix clearly plays an important role, forming a lid over a deep lipid-binding pocket. Consistent with this idea is the observation that this domain always occupies the C-terminal position in mammalian START-proteins containing additional conserved domains, which is essential for flexibility of the C-terminal α helix. Conformational changes governed by membrane contact, protein-protein interactions and/or phosphorylation could account for rapid and efficient lipid transfer between membranes or other donor/acceptor molecules.

Some of the START proteins, such as StAR, are extremely well studied, whereas others, such as STARD8 or STARD9, remain largely uncharacterized. Identification of the ligand specificities and affinities, expression pattern and subcellular localization will be important if we are to understand their functions. In addition, it will reveal how generally applicable the model of START function we favor is to all members of the family.

Another important area of investigation is the role of these proteins in disease. The importance of mutations in *StAR* in lipoid CAH is evident and STARD11 might be implicated in autoimmune pathogenesis. However, the frequent overexpression or loss of START proteins in cancer cells means that the tumor promoting and tumor-suppressor roles of this interesting family should be further explored.

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