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The StarD4 subfamily of steroidogenic acute regulatory-related lipid transfer (START) domain proteins: new players in cholesterol metabolism

Maria Calderon-Dominguez[1], **Gregorio Gil**[2], **Miguel Angel Medina**[3], **William M. Pandak**[4], and **Daniel Rodríguez-Agudo**[4]

[1]Department of Biochemestry and Molecular Biology, Faculty of Pharmacy, Universidad de Barcelona (Spain)

[2]Department of Biochemistry, Virginia Commonwealth University, Richmond, VA

[3]Department of Molecular Biology and Biochemistry, Universidad de Malaga (Spain)

[4]Department of Medicine, Veterans Affairs Medical Center and Virginia Commonwealth University, Richmond, VA

Abstract

Cholesterol levels in the body are maintained through the coordinated regulation of its uptake, synthesis, distribution, storage and efflux. However, the way cholesterol is sorted within cells remains poorly defined. The discovery of the newly described StarD4 subfamily, part of the steroidogenic acute regulatory lipid transfer (START) domain family of proteins, affords an opportunity for the study of intracellular cholesterol movement, metabolism and its disorders. The three members of this intracelular subfamily of proteins (StarD4, StarD5 and StarD6) have a similar lipid binding pocket specific for sterols (cholesterol in particular), but differing regulation and localization. The ability to bind and transport cholesterol through a non-vesicular mean suggests that they play a previously unappreciated role in cholesterol homeostasis.

Introduction

Cholesterol is an essential molecule for the growth and viability of mammalian cells. It is a precursor of different metabolites with important physiological functions, such as vitamin D, bile acids, oxysterols (products of the oxidation of cholesterol) and steroid hormones. Cholesterol is not uniformly distributed in cells, and the maintenance of its distribution is essential for many cellular functions such as cell signaling and membrane trafficking [1]. For example, the endoplasmic reticulum (ER), the main lipid biosynthetic organelle, is sensitive to alterations in cholesterol homeostasis and its membrane has a low cholesterol content [2]. The loss of homeostatic control within the ER leads to ER-stress and triggers the Unfolded Protein Response (UPR) which contributes to the pathology of many human diseases [3].

The intracellular trafficking of cholesterol is meditated by a combination of vesicular and non-vesicular transport pathways [1, 4]. Although prior attention has focused on vesicular

movement, it is becoming increasingly difficult to ignore the non-vesicular trafficking and distribution of cholesterol between organelles not connected to the vesicular transport machinery, such as mitochondria or peroxisomes [5]. Unfortunately, most of the non-vesicular transport mechanisms remain unclear.

In recent years, there has been an increasing interest in the study of a number of specialized non-vesicular lipid transporters, as well as, the steroidogenic acute regulatory-related lipid transfer (START) proteins, involved in the trafficking of lipids and cholesterol between diverse intracellular membranes [6–8]. The proteins of the START domain superfamily are characterized by a lipid binding domain, which appears in a wide range of proteins involved in several cellular functions, including lipid metabolism and transport, transcriptional regulation and signal transduction [9]. Furthermore, phylogenetic analysis divides the family into six subfamilies [9, 10]. It has been proposed that all proteins with a START domain contain a similar binding pocket that binds different ligands based on modification of that binding domain [11]. Using X-ray crystallography, the structures of several proteins with a START domain have been elucidated, including: the StarD4 protein [12] (Figure 1), and recently the structure of the StarD1, StarD5 (Figure 1), StarD13 and StarD14 proteins [13]. All these proteins share the same structure, with the N and C terminal domains separated by several β-strands and two shorter α-helices [11]. The curved β-sheet forms a deep pocket with the C-terminal α-helix acting as a lid, resulting in an internal hydrophobic cavity. The StarD4 structure contains a cavity large enough (~850 $Å^3$) to accommodate a cholesterol ligand (~740 $Å^3$) [9, 10].

In this report we focus in the StarD4 subfamily, and its relevance to cholesterol transport and metabolism. The StarD4 subfamily is composed of three proteins, StarD4, StarD5 and StarD6. In 2002, Soccio and Breslow were able to identify cholesterol-regulated genes in cDNA microarrays of mouse liver. Among them they found StarD4 as novel Expressed Sequence Tags (ESTs). Proteins StarD5 and StarD6 were later identified from a BLAST search of the human genome against StarD4 [10]. Although their functions remain poorly defined, recent studies show that the members of this subfamily are involved in the intracellular trafficking of cholesterol and its cellular homeostasis, as well as, involved in the cytoprotective stage of the UPR in the case of StarD4 and StarD5 [14, 15]. This paper reviews recent research into the structure, regulation and function of the members of the StarD4 subfamily

Structure

The proteins in the StarD4 subfamily have been shown to contain between 205 and 233 amino acids residues, sharing 26–32% identity with each other [10]. Phylogenetically, the StarD4 subfamily is the most closely related to the StarD1 and MLN64 proteins, with ≈ 20% sequence identity. In contrast to StarD1 and MLN64, the proteins of StarD4 subfamily do not have N-terminal targeting sequences that could direct them to specific cellular organelles, therefore, predicted to be cytoplasmic proteins [10].

As mentioned before, StarD4 was initially identified as a sterol-regulated gene [10], and later it was demonstrated that StarD4 mRNA is sterol repressed; consistent with regulation of StarD4 by sterols through the SREBP-2 pathway [16]. New studies showed that not only the StarD4 mRNA responded to changes in the levels of sterols, but the protein as well [17]; indicating the absence of a sterol-mediated post-transcriptional mechanism in the expression of StarD4. Moreover, StarD4 mRNA is also regulated during the early phase of ER-stress. More specifically, the *STARD4* promoter can be activated by the activating transcription factor 6 (ATF6); one of three transcription factors activated during the ER-stress response [14]. This effect has been further supported by studies in differentiating macrophages

showing the maintenance of StarD4 protein levels by the activation of ATF6 (also activated during the differentiation process) despite the inactivation of SREBP2 [17]. The initial studies demonstrated the presence of the StarD4 mRNA in the liver [12]. Most recent studies found StarD4 protein in the hepatic cell line HepG2 [18], and in hepatocytes and non-parenchymal liver cells, like Kupffer cells [17]. At subcellular levels, StarD4 has been found in the cytoplasm and closely associated with the ER in 3T3-L1 cells and THP-1 macrophages [17]. More specifically, in THP-1 macrophages, the protein was found to co-localize with ER-derived vesicles enriched in Acyl-CoA cholesterol acyltransferase-1 (ACAT-1). ACAT-1 catalyzes the intracellular esterification of free cholesterol and was found in close association with lipid droplets [17]. This supports a previous study describing increased levels of cholesteryl esters in hepatocytes following the overexpression of StarD4 [7]. In addition, StarD4 seems to be able to transfer cholesterol to the mitochondria. Studies in hepatocyte cultures showed StarD4's ability to induce steroidogenesis and bile acids synthesis following its overexpression [7, 16].

The second member of StarD4 subfamily is StarD5, the closest related START domain protein to StarD4. StarD5 mRNA expression has been shown to be induced in response to ER-stress, either in free cholesterol loaded mouse macrophages, or in NIH-3T3 and 3T3-L1 cells treated with ER stressors [15, 16]. Despite this, the mechanism that regulates the expression of StarD5 is not completely clear. Recently, two possible mechanisms have been proposed for its regulation under ER-stress. In the first mechanism, StarD5's expression is controlled by the transcriptional factor XBP-1(s) (not by ATF6 or ATF4), whose expression precedes changes in StarD5 mRNA. Furthermore, only XBP-1(s) overexpression was able to induce StarD5 expression, while ATF6 and ATF4 overexpression did not induce StarD5 [15]. A second possible mechanism is based on the posttranscriptional stabilization of StarD5 mRNA during ER-stress. More specifically, under normal conditions the StarD5 mRNA is degraded. However, under ER-stress StarD5's mRNA is stabilized by an unknown signaling in which XBP-1(s) could be implicated as an mRNA stabilization factor. However, this role has not been previously described for XBP-1 [15]. StarD5 protein is expressed in Kupffer cells and in the kidney (proximal tubules, but not in the glomeruli), localizing to the cytoplasm, Golgi and ER membranes [6, 19, 20]. A recent study also revealed not only the expected increase in StarD5 expression under ER-stress in 3T3-L1 cells, but also its protein redistribution from the nucleus and cytosol to the membranes [15].

The third member of StarD4 subfamily is StarD6; likely the member least understood. Its expression has been localized in the nervous system, with the potential for regulation under neurotoxic conditions [10, 21]. StarD6 is also expressed in the testis, but it has not been found in the ovaries; suggesting a special role for StarD6 during male germ cell maturation [10, 22]. Nevertheless, the specific function of StarD6 in spermatogenesis is not yet known. However, recently StarD6 was identified as a putative gene for mitochondrial NADH-dependent dehydrogenase activity (diaphorase), related with sperm quality and motility [23]. StarD6 protein is localized in the cytoplasm and mitochondria [10, 23].

Biological function

Although their biological functions remain poorly defined, recent studies shed some light into their possible roles in the trafficking of cholesterol and in its cellular homeostasis [14, 15].

StarD4 protein, like StarD1 and MLN64, is able to bind cholesterol [6]; but, in addition, has been shown to bind 7-α-hydroxycholesterol and 7-hydroperoxycholesterol [7, 24]. StarD4 overexpression led to an increase in steroidogenesis [24], and in cholesteryl ester formation and bile acid synthesis in primary hepatocytes, denoting an increase in cholesterol transport

to the ER and to the mitochondria [7]. The mentioned primary hepatocytes were cultured under conditions that led to the absence of the CYP7A1 bile acid biosynthetic pathway. Therefore the ability of StarD4 to increase bile acid synthesis was secondary to increased cholesterol transport to mitochondria for synthesis via the “acidic” pathway of bile acid synthesis. Cholesteryl ester formation resulted from increased cholesterol delivery to ACAT-enriched vesicles of the ER for cholesterol esterification and storage [25]. Therefore, StarD4 appears to play a role as a cholesterol transporter between different cellular compartments, facilitating movement from the ER or to other cholesterol carrier proteins. Also, the fact that StarD4 can be regulated by an ER-stress mechanism, during the differentiation of monocytes to macrophages [17], emphasizes the importance of StarD4 in the cell cholesterol metabolism in general and that of the ER in particular. Surprisingly, and despite all the data described above, a newly generated StarD4 K.O. mouse only presented mild changes on lipid metabolism, weight and bile cholesterol in the gallbladder. Therefore, the author suggested that other proteins with similar functions might be able to compensate for the loss of expression of StarD4 [26]. It is also possible that StarD4 is associated with the more transient rapid cholesterol movement; pathways that can be compensated for with more chronic lipid alteration. Interestingly, a recent study in cells where StarD4 was silenced using shRNA showed an increase in cellular free cholesterol as compared to control cells [18] (Figure 2) and changes in NPC-1 and LDL-receptor levels, although more studies are needed to understand these effects.

StarD5 has been shown to bind cholesterol, 25-hydroxycholesterol and the larger fluorescent compound NBD-cholesterol in *in-vitro* assays similarly to StarD1 [6, 27]. While StarD5 overexpression *in-vivo* led to an increase in steroidogenesis [16], *in vitro* assays were not successful [27], questioning the ability of StarD5 to transfer cholesterol inside the mitochondria. These apparent conflicting results could be explained by the requirement of a second transporter; hence StarD5 would deliver cholesterol to the outer mitochondrial membrane, which then is carried to the inner membrane by the second carrier (i.e. StarD1). This possibility however can be questioned based on *in vivo* overexpression assays of StarD5 in hepatocytes, where it induced free cholesterol accumulation in the cells, but it had no effect on bile acid synthesis [6]. Adding more controversy to the issue, recent studies using circular dichroism and nuclear magnetic resonance showed that StarD5 binds primary and secondary bile acids with different affinity depending on the number and positions of the steroid ring hydroxyl groups and the presence or type of conjugation on the side chain of the bile acid [28]. Despite this, the newest studies showing the redistribution of StarD5 protein under ER-stress conditions suggest a role in cholesterol metabolism, where StarD5 could transport cholesterol from the ER to the Golgi/ERC in order to lower the amount of cholesterol that accumulates in the ER during ER-stress (Figure 2).

StarD6 shows an activity analogous to StarD1 with its cholesterol binding and association with the mitochondrial outer membrane; suggesting an important function at the mitochondrial level in male germ cells [29], although its role remains to be clearly defined. StarD6 is also expressed in the nervous system, and although initially was linked to a possible neuroprotective role, [30] this requires further investigation

Possible medical and industrial applications

The concepts reviewed represent novel approaches to further define intracellular cholesterol transport. We suggest that the newly described proteins of the StarD4 subfamily, with their distinctive regulations and localizations, alone or in association with other proteins, play unique and relevant roles in intracellular cholesterol movement and metabolism in a variety of tissues of relevance to human health. Furthermore, future studies on the role of these proteins will give a better understanding of cholesterol metabolism needed to lay the

groundwork for the development of better therapies for cholesterol related disorders (i e. atherosclerosis or Niemann-Pick disease) and UPR related diseases (i.e. Huntington's disease and Alzheimer's disease).

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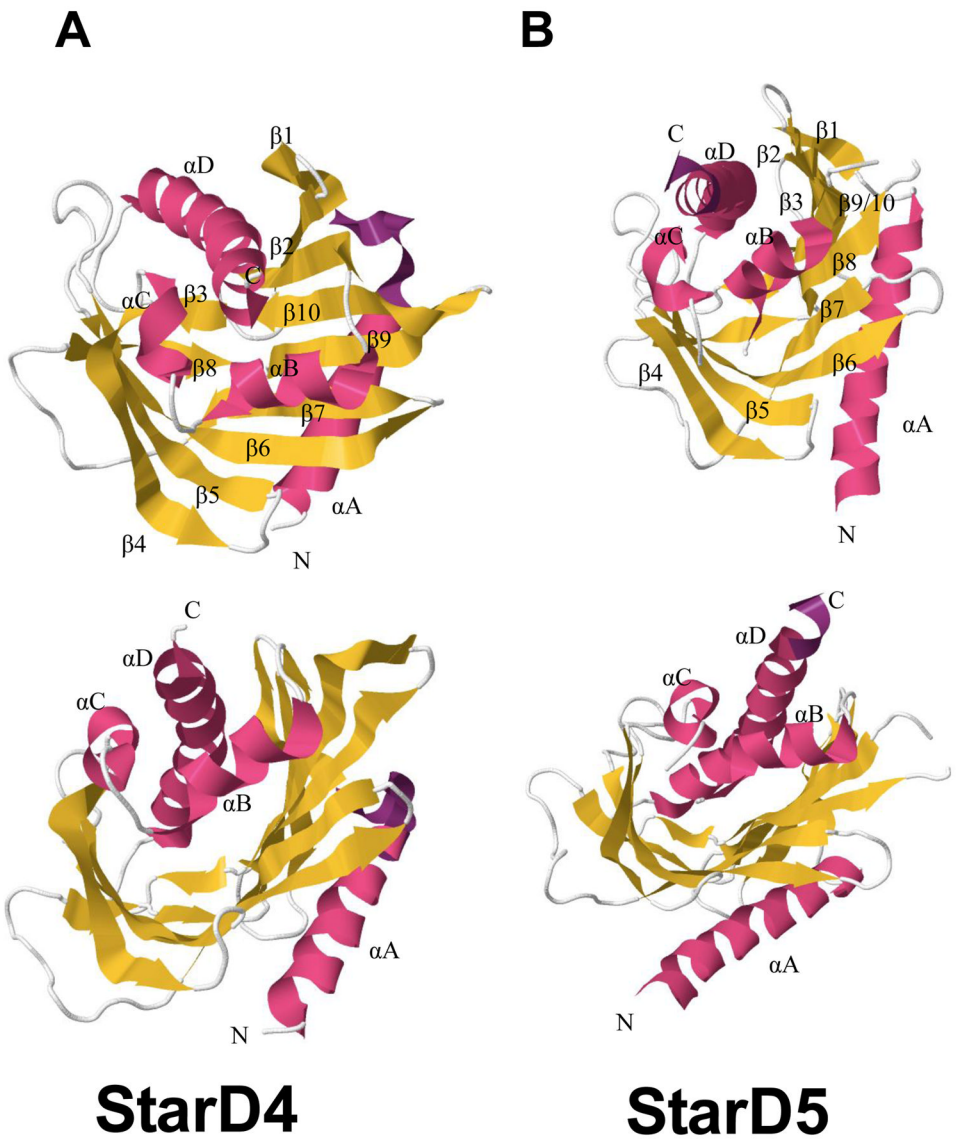

Figure 1.

Cartoons of the secondary structures of mouse StarD4 protein (A; PDB ID: 1JSS) and human StarD5 protein (B; PDB ID: 2R55) indicating all the secondary structures. Images taken of Protein data bank (www.rcsb.org).

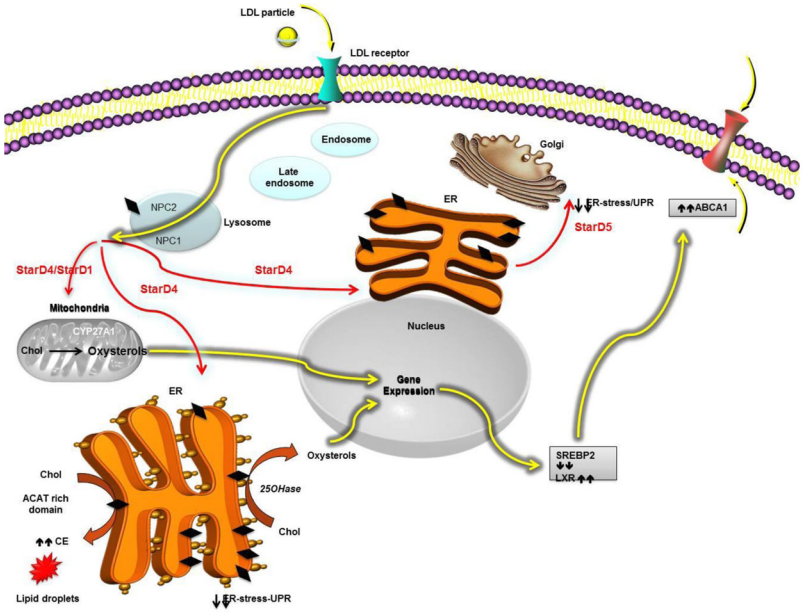

Figure 2.

Schematic representation of intracellular cholesterol transport. A model for StarD4 and StarD5. Highlighted in red are potential functions of StarD4 and StarD5 in the distribution of free cholesterol (◆).

Table 1
Characteristics of the mammalian START 4 domain protein subfamily members

Physical map positions (chromosome, position in megabases, Mb) in the mouse and human genomes are based on the Ensembl database (www.ensembl.org). Cellular location abbreviations used are: endoplasmic reticulum (ER). Lipid binding abbreviations used are: 7-α-hydroxycholesterol (7-α-OHchol), 25-hydroxycholesterol (25OH), cholic acid (CA) and chenodeoxycholic acid (CDCA).

StarD4 subfamily members				
		StarD4	**StarD5**	**StarD6**
Other name (s)		CRSP		
Chromosomme/Position	**Mouse**	18/33.4 Mb	7/73.3 Mb	18/70.8 Mb
	Human	5/110.5 Mb	15/77.6 Mb	18/52 Mb
Subcellular location		Cytosolic[a], ER[a,b], mitochondria[b] [A,B]	Cytosolic, ER, Golgi[a], Nucleus[C–F]	Cytosolic[c], mitochondria[b] [G,H]
Tissue distribution *		Liver, macrophages, keratinocytes, kidney*[A,B]	Kupffer cells, peripheral macrophages, kidney proximal tubules*[D,E]	Nervous system and testis germ cell[G, I–K]
Lipid binding		Cholesterol, 7-α-OHchol, 7-hydroperoxycholesterol #,e[C, L–N]	Cholesterol, 25OH, CA and CDCA d,e[C,O,P]	Cholesterol[d,e] [N]
Regulation		Regulated in response to sterol levels by SREBP pathway[B, Q]. Early phase of ER stress[R]	Induced in response to ER stress [D,F,Q]	Potential regulation under neurotoxic conditions[G,I, J]

*Tissue distribution: Restricted expression, note that STARD4 and STARD5 mRNA have been detected at low levels in heart.

^Cellular location: domains direct subcellular location;

*a*based in immunocyto/histochemistry data for endogenous protein expression;

*b*based on *in vitro* activity;

*c*based in structure.

#Lipid binding: direct ligand binding assay,

*d*modeled based in structure;

*e*based on *in vitro*;

*f*shown in crystal.

References identified by their PMID. A:15976441; B:21767660; C:15897605; D:19474188; E:16534142; F:23053693; G:12011452; H:20209439; I:19434006; J:20609383; K:15564601; L:18403318; M:200559974; N:18211099; O:23872533: P:18250166; Q:15760897; R:16579971. Table based on Clark, B.J.(2012), PMID: 21965545.