COMMENTARY

Cellular itinerary of LDL cholesterol

Jean E. Vance[a,1]

Cholesterol is an essential component of eukaryotic cells. This lipid is synthesized on endoplasmic reticulum (ER) membranes and can also be supplied to cells extracellularly via lipoprotein particles such as low-density lipoproteins (LDLs). These cholesterol-carrying LDL particles bind to transmembrane LDL receptors in the plasma membrane (PM) and are internalized into cells via receptor-mediated endocytosis (1). Mutations in the LDL receptor gene cause the human disease familial hypercholesterolemia in which LDL cholesterol accumulates in plasma, thereby promoting atherosclerosis and heart disease (2). The endocytosed LDL particles are delivered to lysosomes where the LDL cholesterol is released and exported to the PM. Subsequently, the cholesterol is transferred from the PM to the ER, the site at which cellular cholesterol content is tightly regulated by genes encoding several membrane-associated proteins (1). When the cholesterol content of the ER exceeds ~5% of the total lipid mass of the ER, the synthesis of cholesterol and the production of LDL receptors in the ER are down-regulated (3). In addition, excess cholesterol in the ER is esterified to cholesteryl esters for storage in lipid droplets. This stringent regulation of cholesterol homeostasis is essential for normal cell viability and growth. Previous studies by Trinh et al. (4), and from the laboratories of Tontonoz (5) and Saheki (6), have shown that the transport of cholesterol from the PM to the ER in mammalian cells requires the anionic phospholipid phosphatidylserine (PS), as well as the ER- and PM-associated Aster proteins (Fig. 1). However, it was not clear whether PS and the Aster proteins operated together or independently in this pathway. In PNAS, Trinh et al. (7) establish that, although PS and the Aster proteins largely act together in PM-to-ER cholesterol transport, the two factors also partially function independently of one another in this pathway.

The membranes of organelles such as the ER, mitochondria, and PM have defined lipid compositions that are essential for optimal functioning of their membrane-associated proteins. In mammalian cells, cholesterol is highly enriched in the PM compared to the ER. However, the mechanisms by which specific membrane lipid compositions are established and maintained are poorly understood. Most membrane lipids, such as cholesterol and phospholipids, are synthesized on ER membranes and are subsequently transported from their sites of synthesis to other organelle membranes. Consequently, a fundamental challenge is to understand the mechanisms by which these hydrophobic lipid molecules, that are minimally soluble in water, are transported between organelles via the aqueous cytosol. Possible mechanisms proposed for interorganelle lipid trafficking include vesicle-mediated and nonvesicular processes.

Several types of nonvesicular transport have been implicated in the interorganelle trafficking of lipids such as cholesterol (8). One of these proposed mechanisms involves the intermembrane transfer of

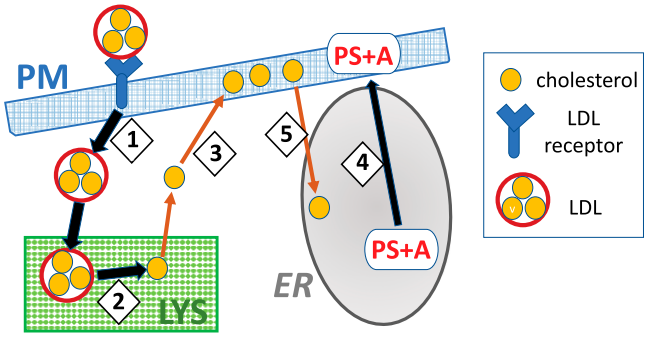

Fig. 1. Movement of cholesterol from the PM to the ER. Cholesterol is synthesized in the ER and is also supplied to cells from extracellular lipoprotein particles such as LDLs. LDLs bind to LDL receptors in the PM and are thereby endocytosed by receptor mediated endocytosis (1). The LDL is delivered to lysosomes (LYS) where cholesterol is released from the LDL (2) and transported to the PM (3). Upon enrichment of the PM with cholesterol, PS and Aster proteins (A) move from the ER to the PM (4) and promote the export of cholesterol from the PM to the ER (5), the site at which cholesterol synthesis and uptake are tightly regulated. Excess cholesterol in the ER is esterified to cholesteryl esters and stored in lipid droplets.

[a]Department of Medicine, University of Alberta, Edmonton, AB T6G 2S2, Canada

Author contributions: J.E.V. wrote the paper.

The author declares no competing interest.

See companion article, "Interplay between Asters/GRAMD1s and phosphatidylserine in intermembrane transport of LDL cholesterol," 10.1073/pnas.2120411119.

[1]Email: jean.vance@ualberta.ca.

Published January 28, 2022.

lipids via soluble cytosolic lipid transfer proteins. Another suggested mechanism for lipid transfer is via the formation of membrane contact sites where the donor and acceptor membranes transiently become closely juxtaposed, as has been widely observed microscopically (reviewed in refs. 9 and 10). The generation of interorganelle membrane contact sites can be facilitated by the formation of reversible protein tethers that link the pair of organelles (reviewed in refs. 9 and 10). In this model of interorganelle lipid transport, the movement of hydrophobic lipid molecules between two organelle membranes could occur without transit of the lipid directly through the aqueous cytosol. In support of this type of mechanism, numerous reports have revealed that ~10% of mitochondria are positioned within 10 nm to 50 nm of elements of the ER. These contact sites between the ER and mitochondria have been reported to mediate the interorganelle transport of PS from its site of synthesis in the ER to mitochondria for decarboxylation to phosphatidylethanolamine, a phospholipid that is essential for mitochondrial function. The membrane contact sites between mitochondrial outer membranes and elements of the ER have been called "mitochondria-associated membranes [MAM]" (11). Similarly, interorganelle contact sites have been detected between the ER and PM, the ER and peroxisomes, the ER and endosomes, and the ER and lipid droplets, as well as between mitochondria and lipid droplets (reviewed in ref. 10). Several of these membrane contact sites have been implicated in interorganelle lipid transport.

Little information is available on the molecular mechanisms by which cholesterol is trafficked between cellular organelles, although the delivery of cholesterol from its site of synthesis in the ER to the PM has been proposed to occur via a nonvesicular process, perhaps involving membrane contact sites. In PNAS, Trinh et al. (7) demonstrate that, when the cholesterol content of the PM increases, the Aster proteins bind PS and form a bridge between the ER and PM, thereby enabling PM-to-ER cholesterol transfer. The delivery of LDL cholesterol from the PM to the ER was assessed by a cholesterol esterification assay, as well as by other measures that monitor the amount of cholesterol in the ER. In combination, these studies demonstrate that both PS and the Aster proteins are required for PM-to-ER transport of cholesterol. A CRISPR-Cas9 knockout screen was performed for genes that are required in Chinese hamster ovary (CHO) cells for the movement of LDL cholesterol from lysosomes to the ER. The major PS-synthesizing enzyme in CHO cells, PS synthase-1 (PTDSS1), was identified as an essential component required for the transport of LDL cholesterol from the PM to the ER. In mammalian cells, PS is synthesized by two distinct PS synthases: PTDSS1 and PTDSS2 (reviewed in ref. 12). Knockout of neither the *Ptdss1* (13) nor the *Ptdss2* (14) gene in mice resulted in any obvious deleterious physiological phenotype, and tissue levels of PS were essentially the same as in wild-type mice. However, the finding that no *Ptdss1*$^{-/-}$/*Ptdss2*$^{-/-}$ double-knockout mice survived demonstrated an essential role for PS and/or its metabolic product phosphatidylethanolamine for mouse viability. In the studies reported by Trinh et al. (7), knockout of the *Ptdss1* gene in CHO cells increased the cholesterol content of the PM and reduced LDL-stimulated cholesterol esterification by 85%, confirming an essential role for PS in the delivery of cholesterol from the PM to the ER. In contrast, knockout of the other PS-synthesizing enzyme, PTDSS2, in human cells did not inhibit this transport process (4). Moreover, despite the ability of PTDSS2 to compensate for PS synthesis in *Ptdss1*$^{-/-}$ mice (13), PTDSS2 was apparently unable to satisfy fully the PS requirement for PM–ER cholesterol transport in *Ptdss1*$^{-/-}$ CHO cells.

In PNAS, Trinh et al. demonstrate that, when the cholesterol content of the PM increases, the Aster proteins bind PS and form a bridge between the ER and PM, thereby enabling PM-to-ER cholesterol transfer.

Knockout of the three Aster proteins also increased the amount of cholesterol in the PM and impaired cholesterol transport from the PM to the ER, as revealed by a 65% reduction in cholesterol esterification. Furthermore, simultaneous knockout of PTDSS1 and the three Aster proteins reduced cholesterol esterification even more dramatically, by 94%. Importantly, in CHO cells lacking the Aster proteins, and also in *Ptdss1*$^{-/-}$ cells, the receptor-mediated uptake of LDL cholesterol and the subsequent transfer of the cholesterol to lysosomes, as well as the delivery of LDL cholesterol from lysosomes to the PM, occurred normally. Similar results were obtained when, instead of the cholesterol content of the PM being increased with LDL, the PM cholesterol pool was increased by incubation of the CHO cells with cholesterol-bound methyl-β-cyclodextrin (which delivers cholesterol directly to the PM). These data demonstrate that the Aster proteins and PS are required for the transport of LDL cholesterol from the PM to the ER.

The finding that the Aster proteins bind PS (as well as cholesterol) (5) suggests that a significant role for the Aster proteins in the export of cholesterol from the PM to the ER might depend upon the binding of PS to Aster. As confirmation of the involvement of PS in the PM–ER movement of cholesterol, Trinh et al. (7) supplied PS liposomes to LDL-treated CHO cells that lacked either PTDSS1 or the Asters, and also supplied PS to cells that lacked both PTDSS1 and the Aster proteins. As monitored by a deficiency in cholesterol esterification and other parameters of cholesterol homeostasis, the reduction in PM-to-ER cholesterol transport in the *Ptdss1*$^{-/-}$ cells, and in the *Ptdss1*$^{-/-}$/*Aster*$^{-/-}$ cells, was reversed by PS supplementation. In contrast, the inhibition of cholesterol esterification was not reversed by the addition of PS liposomes to cells lacking the Asters alone. The data provided by Trinh et al. (7) also show that, although PM-to-ER cholesterol transport requires both the Aster proteins and PS, each of these components possesses additional, independent functions that can partially support the movement of PM cholesterol to the ER.

Several interesting questions arise from these observations. For example, what is responsible for the Aster-independent function of PS in PM-to-ER cholesterol transport? One possibility is that PS is required for an unidentified vesicle-mediated process that promotes PM-to-ER cholesterol transport. A second possibility is that PS is required for the formation of membrane contact sites between the PM and the ER. It is apparent that membrane contact sites exist for many pairs of organelles and that, for at least some of these contact sites, the anionic phospholipid PS promotes formation of membrane contacts. For example, an increase in PS in the MAM domain of the ER enhances the formation of contact sites between the ER and mitochondria, thereby stimulating PS export from the ER to mitochondria (15). Consequently, an intriguing hypothesis is

that PS enrichment of specific membrane domains might be a common feature that underlies the formation of interorganelle membrane contact sites.

It will also be interesting to discover what factors support the residual Aster activity in *Ptdss1*$^{-/-}$ CHO cells. Since some PM-to-ER cholesterol transfer is retained in *Ptdss1*$^{-/-}$ cells, it is possible that the remaining 10% of cellular PS in these cells satisfies this transport requirement. On the other hand, since the Aster proteins can bind anionic lipids other than PS, an alternative anionic phospholipid, such as phosphatidylinositol, might be able to substitute partially for the role of PS in Aster function. Indeed, phosphatidylinositol phosphates can enhance interorganelle lipid transport by promoting the formation of some types of membrane contact sites (16). However, the mechanisms by which cholesterol and phospholipids are transported between organelle membranes remain largely unclear. For example, molecular details of the process by which cholesterol is transported from its site of synthesis in the ER to the PM has not been definitively established. Furthermore, the mechanism by which PS (the synthesis of which is restricted to ER/MAM membranes) is transported to the PM has not been defined.

In summary, the studies reported by Trinh et al. (4, 7) demonstrate that the movement of cholesterol from the PM to the ER requires the action of the PS-binding Aster proteins, as well as a supply of PS. Interestingly, the Aster proteins can partially act in this pathway independently of normal levels of PS; furthermore, some of the functions of PS in this cholesterol transport pathway are independent of the Aster proteins. Future studies are expected to expand our knowledge of these important issues that are relevant to understanding how cholesterol homeostasis is regulated in cells and how these processes might be impaired in human diseases.

1 M. S. Brown, J. L. Goldstein, Receptor-mediated control of cholesterol metabolism. *Science* **191**, 150–154 (1976).
2 J. L. Goldstein, T. Kita, M. S. Brown, Defective lipoprotein receptors and atherosclerosis— Lessons from an animal counterpart of familial hypercholesterolemia. *N. Engl. J. Med.* **309**, 288–296 (1983).
3 A. Radhakrishnan, J. L. Goldstein, J. G. McDonald, M. S. Brown, Switch-like control of SREBP-2 transport triggered by small changes in ER cholesterol: A delicate balance. *Cell Metab.* **8**, 512–521 (2008).
4 M. N. Trinh *et al.*, Last step in the path of LDL cholesterol from lysosome to plasma membrane to ER is governed by phosphatidylserine. *Proc. Natl. Acad. Sci. U.S.A.* **117**, 18521–18529 (2020).
5 J. Sandhu *et al.*, Aster proteins facilitate nonvesicular plasma membrane to ER cholesterol transport in mammalian cells. *Cell* **175**, 514–529.e20 (2018).
6 T. Naito *et al.*, Movement of accessible plasma membrane cholesterol by the GRAMD1 lipid transfer protein complex. *eLife* **8**, e51401 (2019).
7 M. N. Trinh *et al.*, Interplay between Asters/GRAMD1s and phosphatidylserine in intermembrane transport of LDL cholesterol. *Proc. Natl. Acad. Sci. U.S.A.* **119**, e2120411119 (2022).
8 E. Ikonen, X. Zhou, Cholesterol transport between cellular membranes: A balancing act between interconnected lipid fluxes. *Dev. Cell* **56**, 1430–1436 (2021).
9 L. Scorrano *et al.*, Coming together to define membrane contact sites. *Nat. Commun.* **10**, 1287 (2019).
10 J. E. Vance, Inter-organelle membrane contact sites: Implications for lipid metabolism. *Biol. Direct* **15**, 24 (2020).
11 J. E. Vance, Phospholipid synthesis in a membrane fraction associated with mitochondria. *J. Biol. Chem.* **265**, 7248–7256 (1990).
12 J. E. Vance, Historical perspective: phosphatidylserine and phosphatidylethanolamine from the 1800s to the present. *J. Lipid Res.* **59**, 923–944 (2018).
13 D. Arikketh, R. Nelson, J. E. Vance, Defining the importance of phosphatidylserine synthase-1 (PSS1): Unexpected viability of PSS1-deficient mice. *J. Biol. Chem.* **283**, 12888–12897 (2008).
14 M. O. Bergo *et al.*, Defining the importance of phosphatidylserine synthase 2 in mice. *J. Biol. Chem.* **277**, 47701–47708 (2002).
15 M. Kannan, S. Lahiri, L. K. Liu, V. Choudhary, W. A. Prinz, Phosphatidylserine synthesis at membrane contact sites promotes its transport out of the ER. *J. Lipid Res.* **58**, 553–562 (2017).
16 J. Y. Choi, W. R. Riekhof, W. I. Wu, D. R. Voelker, Macromolecular assemblies regulate nonvesicular phosphatidylserine traffic in yeast. *Biochem. Soc. Trans.* **34**, 404–408 (2006).