CURRENT OPINION

LDL–cholesterol transport to the endoplasmic reticulum: current concepts

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Purpose of review
In this article, we summarize the present information related to the export of LDL-derived cholesterol from late endosomes, with a focus on Nieman-Pick disease, type C1 (NPC1) cholesterol delivery toward the endoplasmic reticulum (ER). We review data suggesting that several pathways may operate in parallel, including membrane transport routes and membrane contact sites (MCSs).

Recent findings
There is increasing appreciation that MCSs provide an important mechanism for intermembrane lipid transfer. In late endosome–ER contacts, three protein bridges involving oxysterol binding protein related protein (ORP)1-vesicle associated membrane protein-associated protein (VAP), steroidogenic acute regulatory protein (StAR)D3-VAP and ORP5-NPC1 proteins have been reported. How much they contribute to the flux of LDL–cholesterol to the ER is currently open. Studies for lipid transfer via MCSs have been most advanced in Saccharomyces cerevisiae. Recently, a new sterol-binding protein family conserved between yeast and man was identified. Its members localize at MCSs and were named lipid transfer protein anchored at membrane contact sites (Lam) proteins. In yeast, sterol transfer between the ER and the yeast lysosome may be facilitated by a Lam protein.

Summary
Increasing insights into the role of MCSs in directional sterol delivery between membranes propose that they might provide routes for LDL–cholesterol transfer to the ER. Future work should reveal which specific contacts may operate for this, and how they are controlled by cholesterol homeostatic machineries.

Keywords
lipid transfer, membrane contact site, membrane transport, sterol-binding protein

INTRODUCTION
Cholesterol is a vital constituent of mammalian cell membranes, wherein it regulates membrane biophysical properties and functions of membrane-associated proteins. Cholesterol is heterogeneously distributed between membranes, being enriched in the plasma membrane. In addition, cholesterol is abundant in the endocytic recycling endosomes and in the trans-Golgi-network (TGN) [1]. Instead, the cholesterol content of the ER is low, and it is here that the key regulatory machinery for sensing and adjusting cellular cholesterol levels, the sterol regulatory element-binding protein-SREBP cleavage-activating protein system, is localized [2]. The low ER cholesterol content is achieved by rapid exchange to more cholesterol-rich membranes and by esterification to a fatty acid by acyl-CoA cholesterol acyl transferase, a.k.a. sterol O-acyl transferase, followed by deposition of the produced cholesteryl esters in lipid droplets [3].

Apart from hepatic and central nervous system cells (that are efficient in synthesizing cholesterol de novo), cells acquire cholesterol mainly via receptor-mediated uptake of LDL particles. LDL binds to its receptor at the plasma membrane and is taken up by clathrin-mediated endocytosis. In the acidic pH of endosomes, LDL dissociates from its receptor...
that is recycled to the plasma membrane. LDL in turn is delivered to late endosomal compartments (here referred to as late endosomes) where esterified cholesterol is hydrolyzed by lysosomal acid lipase. The free cholesterol generated is thought to be transferred by the soluble NPC2 protein in the late endosome lumen to the membrane bound NPC1 that inserts it into the late endosomal membrane for export \([4–6]\). What precisely happens after NPC1 has captured cholesterol and how the export of LDL-cholesterol from late endosomes is achieved, is less clear. For instance, the late endosomal membrane protein, LAMP-2, is required for efficient late endosome cholesterol export \([7,8]\), but what its specific role is, remains open.

Biochemical data indicate that two key destinations for the LDL-derived cholesterol are the plasma membrane and the ER. Cholesterol transport to the plasma membrane maintains plasma membrane integrity and functions, whereas transport to the ER is important for ER cholesterol sensing and for providing a backup mechanism of cholesterol storage. With this in mind, several transport routes for LDL-cholesterol from late endosomes to the ER have been proposed: transport first to the plasma membrane and from there to the ER, trafficking from late endosomes to sterol-enriched endomembranes, such as the Golgi, and from there via retrograde transport to the ER and transfer of cholesterol from late endosomes directly to the ER (Fig. 1). The first scenario might help to ensure sufficient cholesterol supply to the plasma membrane prior to

**KEY POINTS**

- LDL-derived late endosomal cholesterol may reach the ER via several routes and membrane compartments, including the plasma membrane.
- The late endosomal compartments receiving LDL-cholesterol form numerous contacts with the ER.
- Several cholesterol-binding proteins localize to late endosome–ER contact sites, but their mode of action is still uncertain.
- A new evolutionarily conserved protein family, Lam proteins, implicated in sterol transfer between ER and late endosomal compartments in yeast, has recently been identified.

**FIGURE 1.** Delivery of LDL-cholesterol to the plasma membrane and endoplasmic reticulum. Left: microscopic images of a cell protrusion showing the positioning of marker proteins Cherry-CD63 [highlighting late endosomes (LEs) and plasma membrane (PM)] and BFP-KDEL [highlighting endoplasmic reticulum (ER)]. Right: simplified schematic illustration of LDL-cholesterol delivery routes. Upon LDL endocytosis, LDL-cholesterol reaches multivesicular bodies (MVBs) and LEs. LDL-cholesterol can reach the PM via a Rab8a/Myo5b/actin-dependent vesicular trafficking pathway or potentially via membrane contact sites (MCSs) via other organelles, such as peroxisomes (P). PM cholesterol can be transported to the ER, potentially involving PM-ER MCSs. LDL-cholesterol can also reach the ER via vesicular trafficking, involving retrograde transport via the Golgi, or may undergo direct transport between LE and ER at MCSs. These MCSs remain to be molecularly characterized but may involve sterol binding proteins (see Table 1). BFP-KDEL was a gift from Gia Voeltz \([44]\).
decreasing cholesterol production in the ER or leading to its sequestration to lipid droplets. This may also apply to the second scenario, as lipid sorting, for example in the TGN can generate cholesterol-enriched carriers targeted to the plasma membrane [9]. On the other hand, an increasing number of examples indicate that sterol transfer can occur via direct late endosome–ER membrane contact sites (MCSSs) to facilitate targeted sterol transfer between late endosomes and the ER [10,11]. In the following, we will briefly discuss these different routings, with emphasis on the most recent literature regarding MCSSs and their potential roles, including new candidate proteins for late endosome–ER cholesterol delivery.

CHOLESTEROL TRANSPORT FROM LATE ENDOSONES TO THE ENDOPLASMIC RETICULUM VIA Plasma Membrane OR TRANS-GOLGI-NETWORK

A major fraction of LDL–cholesterol may initially be transferred to the plasma membrane and surplus plasma membrane cholesterol is then routed to the ER. The interdependence of these routes is supported by observations that LDL–cholesterol loading stimulates the esterification of plasma membrane cholesterol [12,13]. Furthermore, extraction of plasma membrane cholesterol in parallel to stimulation of cholesterol transfer toward the ER results in a drastic reduction of cholesterol reaching the ER [13]. A recent study identified three different cholesterol pools in the plasma membrane: an essential cholesterol pool needed to maintain membrane integrity, a cholesterol pool shielded via interaction with sphingomyelin and releasable upon degradation of sphingomyelin, and a regulatory cholesterol pool that accommodates incoming LDL–cholesterol and can redirect cholesterol to the ER [14]. This three pool-model would enable the plasma membrane to participate in the regulation of cholesterol homeostasis without compromising its major other functions.

How does LDL–cholesterol reach the plasma membrane? We have provided evidence for a Rab8a-dependent membrane recycling route that directs LDL–cholesterol to the plasma membrane, in particular to cell adhesion sites, thereby contributing to cell movement [15]. Late endosome-derived cholesterol carriers apparently fuse with the plasma membrane, but it is possible that this involves prior communication, for example with recycling endosomes. Interestingly, there is also recent evidence for the involvement of late endosome–peroxisome communication in LDL-cholesterol delivery toward the plasma membrane [16].

How plasma membrane cholesterol is transferred back to the ER remains enigmatic. There is evidence that cytoplasmic sterol-binding proteins (in particular, oxysterol-binding protein-related orp proteins in yeast or ORF proteins in mammals) may contribute but are probably not exclusively in charge, as their depletion causes moderate effects on plasma membrane–ER sterol transfer [17–19].

Plasma membrane independent transport of LDL–cholesterol to the ER can be mediated by retrograde membrane trafficking from late endosomes to the TGN. Depletion of key regulators of this pathway reduced cholesterol arrival in the ER [20]. In support for this route, overexpression of Rab9, which regulates late endosome to TGN transport, could rescue late endosomal cholesterol accumulation in NPC1-deficient cells and Rab9 silencing leads to late endosomal cholesterol accumulation [15,21,22]. Cholesterol should then take a retrograde Golgi to ER pathway to reach the ER. In fact, overexpression of Rab6, involved in Golgi-ER transport, can facilitate cholesterol esterification [23]. Notably, the sterol-binding protein oxysterol-binding protein can facilitate cholesterol transport anterogradely from the ER to the Golgi at MCSss [24], but whether a reverse transfer takes place is not clear.

CHOLESTEROL TRANSPORT VIA LATE ENDOSONE: ENDOPLASMIC RETICULUM MEMBRANE CONTACT SITES

Apart from membrane transport, there is increasing evidence for lipid exchange via transfer protein complexes that bridge between two membrane compartments and help to maintain membrane lipid homeostasis. In these MCSs, the neighboring membranes are within a short distance (up to 30 nm), with lipid transfer proteins concentrating at such sites to facilitate directional lipid exchange [10,25]. Accumulating evidence indicates that this transfer can take place as a countertransport of two lipid species, for instance sterol and phosphoinositide [24]. Especially the ER is known to form numerous membrane contacts with other organelles including the Golgi, mitochondria, plasma membrane and endosomes [10,26]. Thus, MCSs between late endosome and ER could potentially allow fast and direct transfer of incoming LDL-derived cholesterol to the ER.

Membrane contacts between the ER and endosomes were visualized by three-dimensional electron microscopy, and live cell microscopy suggested that an astonishing 99% of late endosomes were in persistent association with the ER [27]. Indeed, late endosomes move along ER tubules,
while connections between their membranes seem to be maintained [27,28]. Furthermore, ER–late endosome contacts regulate endosome fission [29], linking sites of endosomal cargo sorting to the ER. So far, two different mechanisms have been proposed for late endosome–ER contact site formation in conjunction with cholesterol sensing or transfer. One involves late endosome–ER tethering via interaction of the ER membrane protein VAP-A with ORP1L or STARD3 in late endosome. The other relies on interaction of ORP5 at the ER side with NPC1 in the late endosome membrane (Table 1).

ORP1L and ORP5 bind cholesterol [32]. ORP1L localizes to the late endosome-limiting membrane and interacts with VAP in the ER, forming contact sites [33] (Table 1). However, the ORP1L/VAP contact site formation is enhanced under cholesterol poor conditions [33,34]. This is counterintuitive as one would expect that if these contacts represent a late endosome–ER LDL–cholesterol transfer route, they would be enhanced when late endosomes are filled by incoming LDL–cholesterol. At present, direct evidence for cholesterol transport at ORP1L/VAP late endosome–ER contacts is lacking, and ORP1L/VAP contacts have been proposed to act as cholesterol sensors rather than actual cholesterol transporters [33].

VAP also generates ER–late endosome contacts via interaction with STARD3, another cholesterol-binding protein embedded in the limiting membrane of late endosome [35,36]. These contacts are independent of ORP1L [37], implying that different sterol-regulated late endosome–ER contacts exist, presumably with different functions. Moreover, ORP1L and STARD3 also target to different subsets of late endosomes, with ORP1L present in more mature late endosomes colocalizing with NPC1, whereas STARD3 localizes to NPC1-negative late endosomes [38]. Overexpression of STARD3 stimulates the formation of late endosome–ER contact sites, and it was suggested that STARD3/VAP contacts are involved in cholesterol transport or cholesterol sensing at late endosome–ER contact sites [37]. However, STARD3 silencing does not result in cholesterol accumulation in late endosomes or disruption of cholesterol transport toward the ER [36]. Also, STARD3 overexpression does not promote cholesterol transport toward the ER [39] but rather toward the plasma membrane, with concomitant cholesterol depletion in the ER [40].

Thus, although the topologies of the cholesterol-binding domains in both STARD3 and ORP1L are correct for sterol export from late endosome, firm evidence for their role in LDL–cholesterol transport to the ER is missing. It is possible that the physiological context in which ORP1L and STARD3 function as cholesterol transporters or sensors is more specialized than that of ‘bulk’ LDL–cholesterol delivery to the ER, but this context has yet to be identified. On the basis of available evidence, it would be equally possible that STARD3/VAP and ORP1L/VAP contacts are involved, for example in the delivery of de-novo synthesized cholesterol from the ER to late endosomes and that expansion of the late endosome–ER contact sites under these conditions might ensure efficient sterol transport.

A third characterized molecular bridge between ER and late endosomes involves the cholesterol-binding proteins ORP5 in the ER interacting with NPC1 in the late endosomal membrane (Table 1). ORP5 depletion was reported to result in late endosomal cholesterol accumulation and inhibit LDL–cholesterol esterification similarly as NPC1, placing it as a potential downstream effector of NPC1 [30]. Yet, the effect of ORP5 silencing on

| Table 1. Late endosome–endoplasmic reticulum contact sites related to cholesterol sensing or trafficking |
|------------------------------------------------------------------------------------------------|
| **Contact site formation via** | **Proof of contact site localization** | **Evidence for cholesterol transport at late endosome–endoplasmic reticulum contacts** |
| ER | LE | |
| VAP-A | ORP1L | Electron microscopy, only VAP-A | – |
| VAP-A | STARD3 | Electron microscopy | – |
| ORP5 | NPC1 | – | ORP5 depletion results in LE cholesterol accumulation and impaired cholesterol esterification [30] |
| Lam6 | | Electron microscopy | Lam6 is required for the formation of sterol-enriched domains in the vacuole. Shifting Lam6 to ER/vacuole contact sites increases vacuolar sterol rich domains [31] |

**Legend:** ER, endoplasmic reticulum; LE, late endosome; NPC1, Nieman-Pick disease type C1; ORP5, oxysterol binding protein related protein 5; ORP1L, oxysterol binding protein related protein 1L; STARD3, Steroidogenic acute regulatory protein; VAP, vesicle associated membrane protein-associated protein.
late endosomal cholesterol accumulation appears less evident than that induced by NPC1 depletion (Pfisterer, Hölttä-Vuori and Ikonen, unpublished observations). Moreover, recent evidence points to a role of ORP5 in the ER–plasma membrane countertransport of phosphatidylserine and phosphatidylinositol phosphate [41*], which needs to be reconciled with the possible late endosome–ER cholesterol transfer function of the protein. Whether ORP5 facilitates cholesterol transport via late endosome–ER contact sites and whether indeed ORP5 and NPC1 are present in late endosome–ER contact sites warrant further investigation.

NEW PROTEINS IN INTERMEMBRANE STEROL TRANSPORT

Recently, a new sterol-binding protein family was identified that was found to localize at MCSs and named the lipid transfer protein anchored at membrane contact sites (Lam) family [42]. It contains three family members in humans and six in S. cerevisiae. So far, most of our knowledge on Lam proteins comes from yeast. Lam2 (Ysp2 is the other name for this protein in yeast), 4 and 6 (lipid transfer at contact site 1) have been shown either to bind sterol or facilitate cholesterol transfer via their conserved START domains [42*]. Interestingly, Lam6 localizes at intracellular MCSs, including ER–vacuole (yeast lysosome) and ER–mitochondria [ER-mitochondria encounter structure (ERMES)] contacts [31*,43*], and Lam6 overexpression can expand MCSs [43*]. The first evidence for the involvement of Lam6 in ER–vacuole sterol transfer comes from observations that Lam6 deletion reduces the formation of sterol-enriched domains in the vacuole. Conversely, Lam6 targeting to ER–vacuole contact sites via disruption of ERMES contacts increases sterol-enriched domains at the vacuole, suggesting that Lam6 stimulates sterol transfer from the ER to the vacuole [31*].

Additional evidence for the involvement of Lam proteins in sterol transfer comes from studies of Lam1 (Ysp1), 2 and 3 (Sip3) that localize to ER–plasma membrane contact sites. Individual deletions of these proteins reduce transport of exogenously added sterols from the plasma membrane toward the ER by up to 50% which is similar to that observed by simultaneous deletion of all Osh proteins [17,42*].

So far, Lam6 is the first example for sterol transfer at ER–vacuole contact sites in S. cerevisiae (Table 1). Given the importance of cholesterol transfer between late endosomes and the ER in mammalian cells, human Lam proteins are emerging as fascinating targets for future investigation. To date, the human Lam proteins hLam-a, b, c (also named GramD1a, b, c) remain uncharacterized. It has been suggested that the START domain of hLam-a has sterol transfer activity in S. cerevisiae [42*], implying that the functions of Lam proteins are evolutionarily conserved. Whereas hLam-a mRNA displays a relatively uniform expression pattern across different tissues, hLam-b transcript is more abundantly expressed in steroidogenic tissue, opening the possibility that it might play a role in cholesterol transfer at ER–mitochondrial contact sites contributing to steroidogenesis.

CONCLUSION

Although NPC1 represents a key gatekeeper protein in LDL–cholesterol transport, there are probably several downstream effectors of NPC1 that may play redundant roles in delivering LDL-derived cholesterol further. For example, no single major pathway of LDL–cholesterol transport to the ER has been identified. There is increasing evidence that MCSs facilitate lipid transfer between juxtaposed membranes. When it comes to late endosome–ER contacts, three molecularly distinct protein bridges involving sterol-binding proteins have been reported: via ORP1L-VAP, STARD3-VAP or ORP5-NPC1 proteins. However, for ORP1L and STARD3-dependent contacts, the available evidence rather disfavors their contribution in LDL–cholesterol delivery to the ER, and for ORP5 contacts, the evidence awaits confirmation. Studies for lipid transfer via MCSs have been most advanced in S. cerevisiae. Although sterol fluxes are somewhat different in yeast because of lack of lipoprotein sterol uptake, the basic mechanisms of lipid transfer are likely conserved. The recent characterization of a novel protein family with members containing a sterol-binding domain and localizing at MCSs has pinpointed the first yeast protein, Lam6, potentially operating in sterol transfer at ER–vacuole contacts. What role(s) the human Lam homologs play in cellular cholesterol delivery will be an interesting new avenue of research.

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Conflicts of interest

There are no conflicts of interest.