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Mechanisms of cellular cholesterol compartmentalization: recent insights

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This review discusses advances in understanding how the controlled delivery of cholesterol between subcellular compartments is achieved and what novel experimental strategies are being employed to address this fundamental question. Recent work has focused on cholesterol-binding proteins that can facilitate directional cholesterol transfer between contacts of the ER and Golgi or late endosomal membranes. Increasing structural information on cholesterol-binding proteins, new modules engineered from them as well as improved imaging and gene editing techniques are providing valuable insights. There is also mounting information on how the crosstalk between cholesterol transport and nutrient signaling is orchestrated and how cellular fatty acid metabolism and cholesterol homeostasis are intertwined.

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Introduction

Cholesterol is an essential component of mammalian cell membranes. It is unevenly distributed between membranes (Figure 1) and rapidly exchanged between them. This article aims to briefly summarize interesting observations made during the past couple of years in the field of cellular cholesterol trafficking and homeostasis, and to point towards emerging trends. Increasing evidence suggests that besides membrane transport, lipid transfer proteins provide specific paths of cholesterol exchange between closely apposed membranes. Advances have been made, for example, in understanding late endosomal cholesterol transport, ER-to-Golgi sterol delivery and links between cholesterol transport, nutrient signaling and fatty acid metabolism. Moreover, improved tools such as biosensors deriving from cholesterol-binding protein modules have been employed for probing cholesterol compartmentalization. For further information on the topic, the reader is referred to more comprehensive reviews [1–5].

Late endosomal cholesterol binding proteins: the usual suspects in cholesterol transport

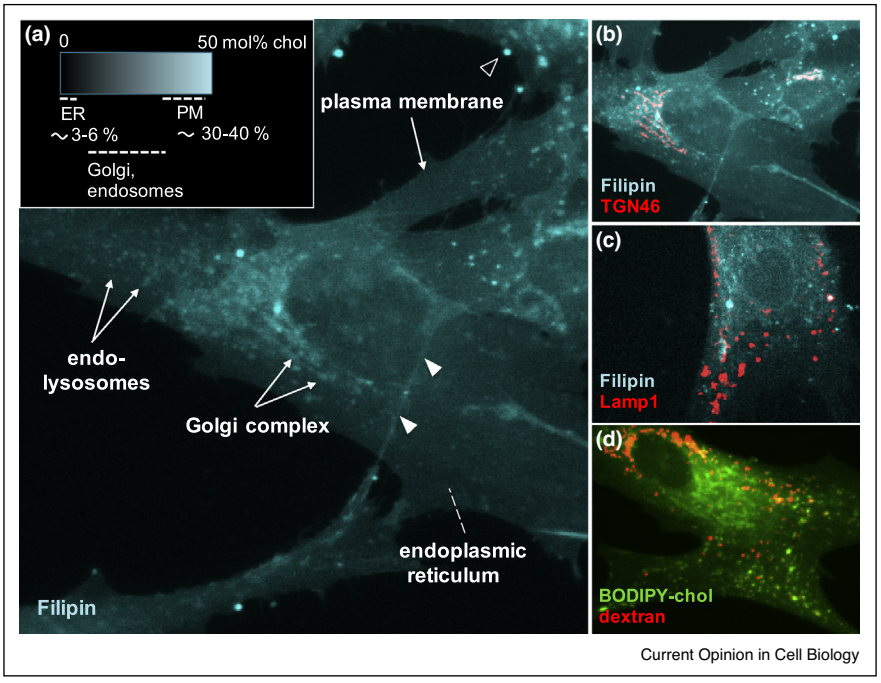
A major route of cholesterol entry into mammalian cells is via endocytosis of low density lipoproteins (LDL). Upon release from the LDL receptor and acid hydrolysis of the particle contents, the released cholesterol is removed from late endosomal organelles via the co-ordinated action of Niemann–Pick type C (NPC) 1 (NPC1) and NPC2 proteins. During the past few years, increasing structural information on NPC1 has revealed insights into the architecture of this complex protein with 13 membrane spans, including luminal interaction with NPC2 and Ebola virus [6–8], membrane embedded sterol-sensing domain [9] and C-terminal luminal domain [10].

Despite these developments, how cholesterol reaches the lysosomal limiting membrane remains mysterious. Cholesterol somehow traverses the ~8 nm thick lysosomal glycocalyx and the heavily glycosylated, abundant lysosomal membrane proteins LAMPs that can also bind cholesterol, play a role [11,12]. Indeed, cholesterol can bind to protein domains located at a considerable distance (12 Å) from the membrane spanning region, as shown, for example, for the extracellular domain of Smoothened [13]. However, also in this case the mechanism by which cholesterol gains access to this binding pocket is unknown.

From lysosomes, LDL-derived cholesterol is delivered to other membrane compartments. Recent findings argue that the bulk of LDL-cholesterol is transported to the plasma membrane, to replenish this cholesterol pool(s) [14,15]. One of the regulators of this route is Rab8a that is recruited in an NPC1-dependent manner to cholesterol-enriched late endosomal organelles and promotes their delivery to the leading edge of the cell [16]. Remarkably, in the absence of functional NPC1, the therapeutic drug candidate β-cyclodextrin can induce subplasmalemmal redistribution of the storage lysosomes and their subsequent release to the extracellular space [17].

The mechanisms of post-NPC1 cholesterol trafficking to the ER remain puzzling, despite the tantalizing presence of cytoplasmically oriented cholesterol-binding and ER interacting domains in late endosomal membrane

Figure 1



Subcellular compartmentalization of cholesterol. Primary human fibroblasts were grown in complete medium and sterol detected by filipin or BODIPY-cholesterol. **(a)** Cholesterol is enriched in the plasma membrane, where it represents about 30–40 mol% of lipids. Plasma membrane protrusions are often cholesterol enriched. Please note a tunneling nanotube connecting two cells (white arrowheads) and plasma membrane derived vesicles/blebs, typical for fibroblast cultures (empty arrowhead). The ER extends throughout the cell but its cholesterol content is low (typically 3–6 mol% of lipids) and not visualized by filipin (dotted line). The cholesterol content of the Golgi complex is intermediate between ER and plasma membrane, increasing in cis-to-trans direction. **(b)** Overlay of filipin staining in A with anti-TGN46 antibody staining. The cholesterol content of endo-lysosomal organelles varies considerably pending on incoming lipoprotein cholesterol uptake. **(c)** Overlay of filipin and anti-Lamp1 staining. **(d)** Cells labeled overnight with dextran and BODIPY-cholesterol. Please note BODIPY-cholesterol perinuclear Golgi-like labeling and partial colocalization with dextran-positive lysosomes.
Source: Images courtesy of Maarit Hölttä-Vuori, stainings and imaging performed as in Refs. [20,37].

proteins [3,4]. In particular, the ORP1L and STARD3 proteins can bind VAP in the ER, bridging between late endosomes and the ER. Interestingly, recent reports provide evidence that both ORP1L and STARD3 transfer cholesterol to the opposite direction, that is, from the ER to endosomes, employing VAP as the ER partner [18•,19•]. STARD3 expression induces cholesterol accumulation in endosomes and favours the formation of pleomorphic membrane structures inside endosomes [19•]. This is in line with earlier findings that dehydroergosterol (DHE) accumulates in late endosomes expressing STARD3 [20].

The ORP1L–VAP interaction is needed for ER-endosome cholesterol transport under low-cholesterol conditions, to support intraluminal vesicle formation in the endosome lumen [18•]. Accordingly, multivesicular body formation was reported to depend on cholesterol and ORP1L [21]. Furthermore, ORP1L–VAP interactions can establish contacts between the ER and late autophagosomes under low-cholesterol conditions [22]. These data fit with the idea that ORP1L–VAP affinity is increased when ORP1L is not sterol bound [23–25]. However, this is not as easy to reconcile with a model where ORP1L mediates cholesterol egress from the endo-lysosomal system under the high endosomal cholesterol conditions induced by LDL loading [26].

Nevertheless, under some conditions ORP1L facilitates cholesterol transfer towards the ER. Upon acute adenoviral infection the viral RIDα protein hijacks the ORP1L sterol-binding domain and apparently induces a novel trafficking route [27]. On the whole, the specialized roles of vesicle subpopulations [28] and dynamic communication between membranes that influences endosome motility, fission, and cargo trafficking are becoming increasingly apparent [29]. In this scenario proteins may not only be recycled but also repurposed, pending on the metabolic cues and local co-incidence of other machinery components.