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# Novel mechanisms of intracellular cholesterol transport: oxysterol-binding proteins and membrane contact sites

Ximing Du, Andrew J Brown and Hongyuan Yang

Cholesterol is an essential membrane constituent, and also plays a key role in cell signalling. Within a cell, how cholesterol is transported and how its heterogeneous distribution is maintained are poorly understood. Recent advances have identified novel pathways and regulators of cholesterol trafficking. Sterol transfer by lipid-binding proteins, such as OSBP (oxysterol-binding protein), coupled with phosphatidylinositol 4-phosphate exchange at membrane contact sites (MCSs) has emerged as a new theme of cholesterol transport between organellar membranes. Moreover, a previously unappreciated role of peroxisomes in cholesterol trafficking has been revealed recently. These discoveries highlight the crucial role of MCSs, or junctions, in facilitating lipid movement, and provide mechanistic insights into how cholesterol is sorted in cells.

**Address**
School of Biotechnology and Biomolecular Sciences, The University of New South Wales, Sydney, NSW 2052, Australia

Corresponding author: Yang, Hongyuan (h.rob.yang@unsw.edu.au)

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## Introduction

Cholesterol is an essential constituent of eukaryotic membranes where it can modulate membrane rigidity, fluidity and permeability [1]. Cholesterol also interacts with membrane scaffold proteins and plays a key role in cell signalling. For example, a recent study demonstrated that cholesterol acts as a *bona fide* regulator of cell signalling and specifically interacts with PDZ domain of the scaffold protein NHERF1 [2•]. The interaction between cholesterol and scaffold proteins may explain why cholesterol-rich membrane domains are crucial for the formation of signalling platforms [3].

Membrane lipids, including cholesterol, are heterogeneously distributed throughout the cell. The uneven distribution of membrane lipids helps to define the identities and properties of cellular compartments, and to maintain proper cell function. Cholesterol is synthesized in the endoplasmic reticulum (ER), which is remarkably cholesterol poor. By contrast, the plasma membrane (PM) is highly cholesterol-enriched, holding almost two-thirds of cellular cholesterol [4]. Cholesterol is also extremely dynamic [5,6] and yet water-insoluble. Given the existence of cholesterol gradients, it has to be sorted and transported efficiently among different intracellular membranes. However, the difficulty of labelling lipid molecules makes it very challenging to fully understand the process of intracellular cholesterol transport [7]. Recently, significant progress has been made in establishing the routes and modes for transport cholesterol between organellar membranes. This mini-review will summarize some of the key recent findings and discuss the molecular mechanisms of both vesicular and non-vesicular transport of cholesterol.

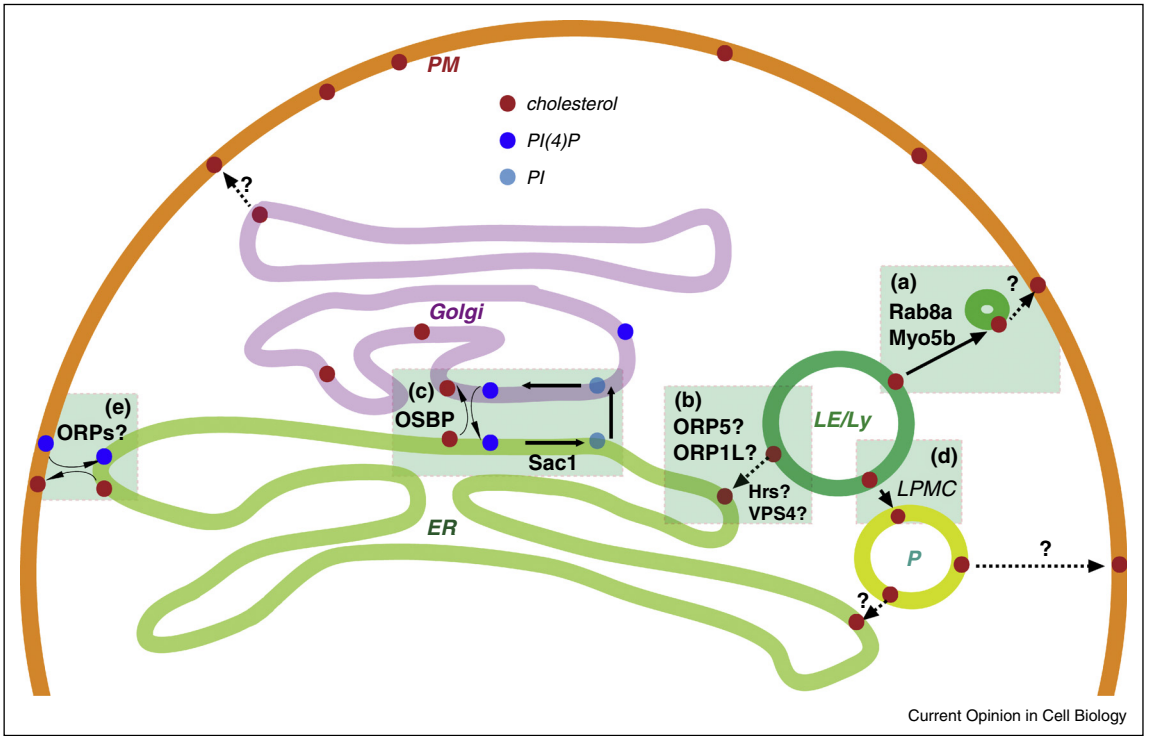
## Vesicular routes for endosomal cholesterol transport

Mammalian cells acquire exogenous cholesterol via uptake of low-density lipoprotein (LDL) through receptor mediated endocytosis [8]. In late endosome/lysosome (LE/Ly), cholesteryl esters carried by LDL are hydrolyzed by acid lipase to released free cholesterol. Inside the lumen of LE/Ly, a soluble cholesterol-binding protein NPC2 (Niemann–Pick Type C 2) [9], accepts and delivers LDL cholesterol to NPC1, a LE/Ly membrane protein [10], and the N-terminal domain of NPC1 binds and inserts LDL cholesterol directly into LE/Ly membrane for export [11–15]. LDL cholesterol exits LE/Ly membrane and travels to the ER, PM and other cellular compartments for regulatory and structural roles [16,17]. For example, once reaching the ER from LE/Ly, LDL cholesterol inhibits the sterol regulatory element-binding protein (SREBP) pathway, subsequently decreasing cholesterol synthesis and uptake [18]. LDL cholesterol in the ER also blunts cholesterol synthesis by accelerating the proteasomal degradation of key enzymes including 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase [19] and squalene monooxygenase [20]. Furthermore, LDL cholesterol increases the activity of ACAT1, which converts free cholesterol into cholesteryl esters that are stored in cytosolic lipid droplets [21]. Therefore, post-LE/Ly transport of LDL cholesterol is of crucial importance to the regulation of cellular cholesterol homeostasis. To this end, it is well-established that LE/Ly membrane protein NPC1 is essential for the efficient release of cholesterol from LE/Ly, but no molecular details are available for the post-NPC1 trafficking of cholesterol.

A recent study provided insights into how LDL cholesterol is recycled back to the PM [22••] (Figure 1). Using LDL labelled with BODIPY cholesteryl linoleate, Ikonen and co-workers were able to record high-resolution time-lapse images of post-endosomal sterol transport in living cells [22••]. Like native LDL cholesteryl ester, the labelled LDL was efficiently hydrolyzed by lysosomal acid lipase and mobilized by NPC1. They identified Rab8a as an important regulator of post-NPC1/endosomal transport of LDL cholesterol to the PM [22••]. The delivery of LDL cholesterol to the PM was impaired when Rab8a or its upstream interactors, ARF6 and Rabin8, were depleted. Rab8a was apparently recruited to BODIPY cholesterol-enriched LE/Ly upon LDL loading in an NPC1-dependent manner. Once re-directed to LE/Ly, Rab8a facilitated the movement of BODIPY cholesterol-containing vesicles from LE/Ly to the PM. This vesicle transport required microtubules and actin and was mediated by Myo5b [22••]. However, the precise mechanisms by which Rab8a–Myo5b complex is associated with LDL cholesterol-containing vesicles and how the vesicles interact with the PM to deliver cholesterol remain to be elucidated. Nevertheless, this work, together with earlier studies [23,24], suggested the involvement of vesicular transport in endosomal cholesterol trafficking.

It is worth noting that intraluminal vesicles in the endocytic pathway may also be required for cholesterol egress from LE/Ly. Recently, two important proteins related to the endosomal sorting complex required for transport (ESCRT) pathway have been shown to play essential roles in the delivery of LDL cholesterol to the ER [25•,26•]. ESCRT proteins cooperate to generate intraluminal vesicles at multivesicular bodies, and mediate the degradation of ubiquitinated membrane proteins [27–29]. Hrs, an ESCRT-0 component, and VPS4, an AAA ATPase well-known for its role in ESCRT-III disassembly, appeared to regulate the efflux of cholesterol from endosomes through distinct mechanisms [25•,26•]. Depletion of Hrs or VPS4 (both VPS4A and VPS4B isoforms) led to the accumulation of LDL cholesterol in LE/Ly and dysregulation of cellular cholesterol homeostasis in the

**Figure 1**




A simplified model of intracellular cholesterol trafficking. **(a)** Cholesterol is transported from late endosome (LE)/lysosome (Ly) to the plasma membrane (PM) via vesicles mediated by Rab8a/Myo5b. **(b)** ORP5, ORP1L, Hrs, and VPS4 may be involved in cholesterol transport from LE/Ly to the endoplasmic reticulum (ER). **(c)** Cholesterol/PI(4)P exchange by OSBP between the ER and *trans*-Golgi. OSBP serves as a tether at membrane contact sites between the ER and *trans*-Golgi, where it extracts cholesterol from the ER membrane and transport it to the *trans*-Golgi. After cholesterol is released in the *trans*-Golgi, PI(4)P is extracted by OSBP and transported to the ER, where it is hydrolyzed by Sac1 in the ER. Thus, cholesterol/PI(4)P exchange is fulfilled by the OSBP cycle. **(d)** A lysosome–peroxisome (P) membrane contact (LPMC) formed by Syt7 binding to PI(4,5)P2. **(d,e)** Whether OSBP/ORPs play any role in cholesterol transport between the ER and the PM or at the LPMC is unclear. OSBP, oxysterol-binding protein; ORP5, OSBP-related protein 5; ORP1L, OSBP-related protein 1 long form; Myo5b, myosin 5b; Hrs, hepatocyte growth factor regulated tyrosine kinase substrate; VPS4, vacuolar protein sorting-associated protein 4; PI(4)P, phosphatidylinositol 4-phosphate; PI, phosphatidylinositol.