Sterol and lipid trafficking in mammalian cells

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Abstract
The pathways involved in the intracellular transport and distribution of lipids in general, and sterols in particular, are poorly understood. Cholesterol plays a major role in modulating membrane bilayer structure and important cellular functions, including signal transduction and membrane trafficking. Both the overall cholesterol content of a cell, as well as its distribution in specific organelar membranes are stringently regulated. Several diseases, many of which are incurable at present, have been characterized as results of impaired cholesterol transport and/or storage in the cells. Despite their importance, many fundamental aspects of intracellular sterol transport and distribution are not well understood. For instance, the relative roles of vesicular and non-vesicular transport of cholesterol have not yet been fully determined, nor are the non-vesicular transport mechanisms well characterized. Similarly, whether cholesterol is asymmetrically distributed between the two leaflets of biological membranes, and if so, how this asymmetry is maintained, is poorly understood. In this review, we present a summary of the current understanding of these aspects of intracellular trafficking and distribution of lipids, and more specifically, of sterols.

Introduction
Eukaryotic cells maintain a high rate of vesicular membrane traffic involving their secretory and endocytic organelles and the plasma membrane (see Figure 1). The best quantitative analyses of the rates of membrane traffic involve the plasma membrane, and it has been estimated that an amount of membrane equivalent to the entire cell surface is internalized with a t½ of 15–30 min in cultured fibroblasts [1]. From the cell surface, internalized membrane constituents can be delivered to a variety of different destinations, including recycling to the cell surface, delivery to late endosomes and lysosomes, and delivery to the Golgi apparatus [1,2]. In some cases, molecules are even transported in a retrograde manner through the Golgi and back to the ER (endoplasmic reticulum) [1,2]. With such a high rate of vesicular membrane traffic and so many interconnecting pathways of trafficking, it is challenging to understand how the cell correctly sorts membrane constituents so that each organelle maintains its distinct composition of membrane proteins and lipids.

The membrane trafficking of proteins has been studied extensively, and the mechanisms for membrane protein sorting are becoming well understood even at the molecular level [3–5]. In general, certain cytosolic/peripheral membrane proteins with adaptor/facilitator functions associate with the machinery for forming a vesicle or tubule bud from a donor membrane. Some transmembrane proteins associate with these adaptor/facilitator proteins either directly or indirectly, and consequently, the whole complex becomes concentrated in the forming vesicles or tubules. Usually, the protein–protein associations responsible for sorting involve the cytoplasmic domains of cargo proteins, and often short cytoplasmic peptide sequence motifs are sufficient to facilitate inclusion in a forming vesicle bud [1,2]. In addition to this signal-mediated sorting, highly efficient protein sorting can occur in some cases by physical mechanisms that are based on the properties of the organelles and on general properties of the cargo proteins [1,2]. For example, shortly after internalization via coated pits, many receptors and their ligands are delivered to a mildly acidic, tubulo-vesicular organelle called the sorting endosome (Figure 1). Many ligands [e.g. LDL (low-density lipoproteins)] are dissociated from their receptors by the acidic pH in the endosome. When narrow tubules bud off from the sorting endosomes, they have a high surface area to volume ratio compared with the more spherical centre of the organelles. As a result, in the absence of other sorting signals, membrane proteins (e.g. LDL receptors) will be

Key words: cholesterol, dehydroergosterol, endocytic recycling compartment, lipid, membrane trafficking, sterol carrier protein (SCP).
Abbreviations used: CHO cell, Chinese-hamster ovary cell; DHE, dehydroergosterol; ER, endoplasmic reticulum; ERC, endocytic recycling compartment; LDL, low-density lipoproteins; ORP, oxysterol-binding protein-related protein; SCP, sterol carrier protein; StAR, steroidogenic acute regulatory protein; STARD, STARD-domain-containing protein; START, START-related lipid transfer.

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Figure 1 | A schematic representation of intracellular cholesterol transport

Cholesterol and cholesteryl ester bound to LDL are internalized by LDL receptors and transported to sorting endosomes. Low pH of sorting endosomes allows separation of LDL receptor from its cargo and the receptor is recycled back to the plasma membrane via the ERC. Free LDL is transported to late endosomes and lysosomes from which cholesterol can efflux to cellular compartments, including the plasma membrane and ER. Cholesterol efflux from late endosomes and lysosomes is poorly characterized, as indicated by broken lines. Cholesterol can move from the plasma membrane to the ERC by a non-vesicular, ATP-independent process. Recycling of cholesterol back to the plasma membrane occurs partly by vesicles carrying other recycling membrane components, but non-vesicular pathways are also important as shown by the broken line. De novo-synthesized cholesterol in the ER is mostly transported from the ER directly to the plasma membrane by non-vesicular processes, although some of it follows the biosynthetic secretory pathway from the ER to the Golgi and then to the plasma membrane. Excess cholesterol in the ER is esterified by ACAT (acyl-coA:cholesterol acyltransferase) and the esters are stored in cytoplasmic lipid droplets. Free cholesterol can move directly to lipid droplets from the plasma membrane. The $t_{1/2}$ values for the various pathways are discussed in [1,18,30,32].

Intracellular trafficking of lipids

In addition to proteins, the lipid constituents of the membrane are also sorted during membrane trafficking processes [6–12], although the mechanisms for their vesicular sorting are not as
well understood as that of proteins. In few cases, lipids may be sorted by specific binding to proteins [2], but it is likely that most sorting of lipids in vesicle trafficking occurs by physical mechanisms involving the properties of the lipids and the properties of the parent organelle and the buds that form from them. In studies with fluorescent lipid analogues, it has been shown that after endocytosis lipids can be sorted to different organelles based in part on their preferences for different types of membrane organization [13,14]. Furthermore, the sorting of lipids in the endocytic pathway can be altered by reducing cellular cholesterol levels or by increasing the amount of unsaturated lipids in the membrane [15]. Both of these treatments would be expected to alter the formation of lipid microdomains, and the effects suggest that lipid preferences for various types of microdomains may affect their inclusion into forming vesicle or tubule buds [9]. This type of sorting would help to maintain distinct lipid compositions in different organelles even in the presence of extensive mixing by vesicle traffic.

Two additional issues must be considered in lipid trafficking as compared with membrane protein trafficking. The first is the transbilayer distribution of the lipids, and the second is the role of non-vesicular trafficking. Most biological membranes maintain a compositional asymmetry across the bilayer, and this is maintained by active flipping of certain lipids by processes that are not well characterized at the molecular level [16]. The existence of metabolic energy-independent flipases for transbilayer translocation of lipids has also been postulated, but these are yet to be identified [16,17]. Lipids on the cytoplasmic leaflet can, in principle, leave the bilayer, diffuse through the cytoplasm, and be reincorporated into a different bilayer. However, in the absence of carrier proteins, such processes would be extremely slow due to the poor solubility of the hydrophobic chains in the aqueous environment. In such non-vesicular transport, proteins play three key roles: extracting the lipid from the donor membrane, providing a hydrophobic pocket for the lipid during transport, and facilitating insertion into the acceptor membrane. Specificity of a carrier protein for a certain transport step can be achieved in one of several ways. It could bind to selected donor or acceptor organelles by protein–protein or protein–lipid interactions. It could also have selectivity for extracting and carrying certain types of lipids based on the properties of the binding pocket.

In the present review, we focus primarily on the intracellular trafficking of lipids, and in particular that of cholesterol, with special emphasis on the non-vesicular component of their itineraries.

**Distribution and trafficking of sterols in mammalian cells**

Cholesterol plays an important role in the organization of lipid bilayers, and disorders related to the transport or distribution of cholesterol can have severe consequences for cells and for organisms [18]. An important function of cholesterol, in association with sphingolipids, is to organize lipids into a liquid-ordered structure (also known as rafts) that provides a high degree of order within the bilayer while permitting rapid lateral diffusion within the bilayer [9,19]. The liquid-ordered lipid organization can co-exist in a bilayer with other types of lipid organization, especially liquid-disordered regions, in which the acyl chains are less tightly packed. The amount of cholesterol in a membrane is an important determinant of the bilayers’ physical properties, and the level of free (non-esterified) cholesterol is tightly regulated at the cellular and organismal level [9,19].

The levels of cholesterol vary greatly among cellular organelles. In cultured fibroblasts, the plasma membrane and the ERC (endocytic recycling compartment) have the highest levels of cholesterol, and it has been estimated that on a molar basis, approx. 30% of the lipids in the plasma membrane are cholesterol [20,21]. The ER is the site of cholesterol synthesis, but its membranes have a low cholesterol content [22]. Within a membrane bilayer, cholesterol is not necessarily distributed homogeneously. Localized liquid-ordered domains (rafts) may have higher cholesterol concentrations than surrounding liquid-disordered domains, but this concentration difference has not been documented in biological membranes. There is some evidence in model membranes that cholesterol is only slightly enriched in ordered domains as compared with co-existing liquid-disordered membranes [23,24]. The transbilayer distribution of cholesterol in biological membranes is also uncertain. Cholesterol can flip rapidly between the leaflets in a bilayer, and it has been shown that the $t_{1/2}$ for this is less than a second in erythrocytes [25]. Some models propose preferential interactions between cholesterol and sphingomyelin (which is predominantly on the outer leaflet of membranes) [26,27], but studies of the transbilayer distribution of fluorescent sterols have indicated that most of the sterol is in the inner leaflet of human erythrocytes [28].

In our laboratory, we have studied the transport of DHE (dehydroergosterol), a naturally occurring fluorescent sterol that has many properties similar to cholesterol. We found that the subcellular distribution of DHE is very similar to cholesterol [29,30]. Like cholesterol, DHE can support the formation of liquid-ordered domains, and a large fraction of cholesterol and DHE in cells is resistant to solubilization by cold Triton X-100 [31]. When DHE esters are incorporated into lipoproteins, the esters are hydrolysed and the free sterol is released into the cell with kinetics similar to cholesteryl esters [32]. Additionally, the DHE can be re-esterified by cells similarly to the esterification of cholesterol [32]. All of these results suggest that DHE is a good analogue of cholesterol.

The intracellular transport of sterol has been studied using radioactive labels and fluorescent sterols, and these results indicate that there is a constitutive high rate of sterol transport in cells. Using radioactive biosynthetic precursors of cholesterol, DeGrella and Simoni [33] showed that newly made cholesterol could be delivered from the ER to the plasma membrane with a $t_{1/2}$ of approx. 10 min. When CHO (Chinese-hamster ovary) cells were labelled with DHE, the DHE in the ERC could be selectively photobleached, and
the rate of recovery of fluorescence in the ERC provides a measure of the rate of transport of DHE into the ERC. It was found that the rate of recovery of fluorescence in the ERC was approx. 2.5 min [30]. This indicates that approx. \(10^6\) molecules of sterol can be transported into the ERC per second (see the Appendix).

How do sterols move within cells? One obvious possibility is that they move as part of the membrane in vesicular transport processes. It is clear that some sterol does move this way [20], but there is substantial evidence that this is not the major mechanism for intracellular sterol transport. In fibroblastic BHK (baby hamster kidney) cells, treatment with Brefeldin A, which blocks vesicular transport out of the ER, only reduces the rate of transport of newly synthesized cholesterol from the ER to the plasma membrane by approx. 20\% [34]. Similarly, in yeast cells with a defect in the Sec18 protein, which is essential for most vesicle traffic out of the ER, transport of ergosterol from the ER to the plasma membrane is unaffected [35]. When the transport of DHE into the ERC was measured by photobleaching recovery, it was found that the rate of recovery was not affected by ATP depletion of the cells, and the extent of recovery was only slightly reduced [30]. Since ATP is required for vesicular transport, this indicates that most of the delivery to the ERC is non-vesicular. Similar results were found for the transport of DHE to lipid droplets [32]. All of these results indicate that the major mode of intracellular sterol transport is non-vesicular, although vesicular transport may play an important role in some sterol transport processes.

**SCPs (sterol carrier proteins) in non-vesicular sterol transport**

Several proteins have been proposed to serve as cytoplasmic carriers of cholesterol. These include cytoplasmic SCP-2, the ORP (oxysterol-binding protein-related protein) family, START [StAR (steroidogenic acute regulatory protein)-related lipid transfer] domain proteins and caveolins. Definitive evidence for the roles played by these proteins in most sterol transport processes is generally lacking.

SCP-2 is a soluble lipid transfer protein which is capable of cholesterol transport in vitro [36]. The rate of transfer of newly synthesized cholesterol to the plasma membrane was decreased in SCP-2-deficient cells [37] and increased after overexpression of SCP-2 [38]. A role for caveolin in cholesterol transport has been proposed in several studies [39,40]. In cultured fibroblasts, reduction in caveolin expression by antisense DNA reduces cholesterol efflux, whereas transfection with caveolin cDNA stimulates this process [41]. It has been proposed that caveolin forms a chaperone complex with cholesterol and that these intracellular lipid particles transport cholesterol [42,43]. However, some studies have failed to find evidence that this cholesterol–chaperone complex participates in surface delivery of newly synthesized cholesterol [34].

Overexpression of ORP2 in HeLa and CHO cells increased efflux of cholesterol to cyclodextrin without disturbing the total plasma membrane pool, suggesting a possible role of ORP2 as a carrier protein [44]. Structural studies of an ORP are consistent with a role in trafficking of cholesterol [45]. Hurley and co-workers [45] have shown that a single sterol molecule binds within a hydrophobic tunnel of Osh4, a yeast ORP protein, in a manner consistent with a transport function for Osb4. Mutations of basic residues within the tunnel abrogate both cholesterol binding and biological function.

An extensive family of proteins containing START domains have been shown to bind lipids, including sterols, and play a significant role in intracellular lipid transport. The prototype is StAR1, which transfers cholesterol from the outer to the inner mitochondrial membrane in steroid hormone-producing cells, but the precise mechanism of StAR-induced cholesterol import into mitochondria is not well understood [46]. Although structural studies of STARD1 (StAR-domain-containing protein 1), STARD3 and STARD4 support the binding of cholesterol to these proteins, their direct role in sterol movement inside the cell is still speculative [47,48].

There are several important issues to consider in analysing the function of these potential sterol carriers. First, together, they must be able to move a large number of cholesterol molecules per second. We have estimated that approx. \(10^6\) molecules of sterol enter the ERC per second. Presumably, a comparable number of molecules enter and leave the plasma membrane per second. The transport proteins may carry targeting information to selectively deliver sterol to specific organelles. However, using DHE, we found that most of the sterol would be delivered to the ERC in preference to other internal organelles in permeabilized cells even by a cyclodextrin carrier which would lack any organelle targeting capability [30]. This presumably indicates that the cytosolic leaflet of the ERC is maintained in a state such that it is kinetically or thermodynamically the best sterol acceptor among all of the organelle membranes. Further work will be required to understand how this works.

The combination of new genetic approaches for manipulating cells and the development of fluorescent tools for analysing lipid and sterol trafficking make this an exciting time to be studying vesicular and non-vesicular transport of lipids. It is hoped that in the next few years, specific roles can be assigned to many of the potential sterol transport proteins and, consequently, a better understanding of how the distribution of cholesterol among organelles is maintained will emerge.

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**Appendix**

**An estimate of the rate of sterol influx into the ERC of a typical mammalian cell**

The following is a rough estimate of the rate of cholesterol transport into the ERC based on our measurement of the transport rate constant.
First, we assume that a typical mammalian cell is a sphere of diameter 10 μm. Thus its surface area will be approx. 3 × 10^8 nm². We then assume that approx. 2/3 of this membrane surface area is occupied by lipids (the rest will be occupied by proteins). The average surface area of a lipid molecule is 0.6 nm² [49]. Thus the plasma membrane will contain roughly 10^10 lipid molecules in both leaflets.

If sterol comprises approx. 30% of the total plasma membrane lipids [20], then the plasma membrane will contain approx. 3 × 10^8 sterol molecules. If we assume that 50% of the cell sterol is at the plasma membrane, then the whole cell will have 6 × 10^9 sterol molecules. A recent study from our laboratory suggests that the ERC membrane contains approx. 40% of the total cellular sterol [30]. Thus the number of sterol molecules in the ERC at any time will be approx. 2 × 10^8.

Finally, we have shown that if the ERC is photobleached, the half-time of refilling it with sterol is 150 s [30], so the first-order rate constant for this process is approx. 0.005. Thus approx. 10^8 molecules of sterol enter and leave the ERC per second.

References

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