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Review

Where sterols are required for endocytosis

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Abstract

Sterols are essential membrane components of eukaryotic cells. Interacting closely with sphingolipids, they provide the membrane surrounding required for membrane sorting and trafficking processes. Altering the amount and/or structure of free sterols leads to defects in endocytic pathways in mammalian cells and yeast. Plasma membrane structures functioning in the internalization step in mammalian cells, caveolae and clathrin-coated pits, are affected by cholesterol depletion. Accumulation of improper plasma membrane sterols prevents hyperphosphorylation of a plasma membrane receptor in yeast. Once internalized, sterols still interact with sphingolipids and are recycled to the plasma membrane to keep an intracellular sterol gradient with the highest amount of free sterols at the cell periphery. Interestingly, cells from patients suffering from sphingolipid storage diseases show high intracellular amounts of free cholesterol. We propose that the balanced interaction of sterols and sphingolipids is responsible for protein recruitment to specialized membrane domains and their functionality in the endocytic pathway.

Keywords: Sterol; Endocytosis; Sphingolipid; Membrane domain; Plasma membrane; Membrane transport

Contents

1. Introduction

Interaction with the surroundings is one of the most remarkable features of life. To respond properly to outside stimuli, eukaryotic cells internalize extracellular material along with parts of their own limiting membranes in a process termed endocytosis. Particulate and water-soluble

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nutrients, signaling molecules, and portions of plasma membrane are taken up by cells in an energy-dependent manner and transported along the endocytic pathway to a degradative organelle. Some components, especially plasma membrane proteins and lipids, escape degradation and are recycled to the plasma membrane.

Biochemical analysis in mammalian cells and yeast as well as genetic screens in *Caenorhabditis elegans* and the yeast *Saccharomyces cerevisiae* have identified not only the compartments of the endocytic pathway but also the molecular requirements for this membrane transport route [1]. The endocytic machineries in mammalian cells and yeast show a high degree of conservation in the eukaryotic world; however, differences exist. These concern not only internalization signals of cell surface receptors [2,3], but also the coupling of endocytic cargo to vesicle coats [4,5] and, in particular, the role of the actin cytoskeleton and its associated proteins [6]. Whereas in *S. cerevisiae* the actin cytoskeleton is of major importance in endocytosis, its contribution to clathrin-mediated endocytosis in animal cells is less predominant. Post-internalization steps of endocytosis require several families of structurally and functionally homologous proteins in yeast and mammalian cells, e.g. SNARE proteins and small GTPases of the Rab/Ypt family [7,8].

The requirements for proteins and their specific functions in endocytosis have been accepted by the scientific community for some time [9,10]. The second main component of biological membranes, lipids, on the other hand, more recently gained the status of active players in the endocytic pathway. We are just beginning to unravel their roles in membrane transport. Various lipid classes are involved, e.g. glycerophospholipids, sphingolipids and sterols, offering a wide range of molecular properties and functional groups. Lipids form the proper hydrophobic environment for proteins containing transmembrane domains and they are responsible for membrane attachment of proteins by GPI-anchors, acylation and prenylation [11–13]. Furthermore, lipids function in signal transduction [14,15] and can be modified, e.g. phosphorylation of phosphatidylinositol, to yield an array of regulators for adaptor binding [16] and binding of other proteins to membranes. In this review, we focus on the role(s) of sterols in endocytosis. Excellent recent reviews have summarized the current knowledge on endocytosis and the functions of other lipid classes therein [17–22]. We will summarize evidence for the importance of a balanced interaction between sterols and proteins as well as other lipid classes in the endocytic pathway.

2. Sterol synthesis and structures

Sterol synthesis proceeds via very similar pathways in mammalian cells and yeast and has been extensively reviewed elsewhere [23,24]. The structural differences between cholesterol and the main sterol of yeast, ergosterol, are highlighted in Fig. 1. Ergosterol contains one more methyl group in the side chain at position C-24 and two additional double bonds at C-7 and C-22. These alterations might not seem dramatic, but biophysical experiments have documented clear effects in artificial membrane systems [25].

3. Compartments of the endocytic pathway and their lipids

The plasma membrane is the donor membrane for the formation of endocytic vesicles and it has a most outstanding lipid composition. Of all the membranes in eukaryotic cells, the plasma membrane has the highest concentration of free sterols [26,27]. Generally, sterols are synthesized in the endoplasmic reticulum (ER) and are then found more concentrated at each step of the secretory pathway, from the ER to the Golgi compartment and to the plasma membrane [28]. However, in most animal cells significant amounts of sterols are released from endocytosed low-density lipoproteins (LDL) [29,30]. Sterols have an intrinsic tendency to interact intimately with sphingolipids, which are also abundant in the plasma membrane [31,32]. It is speculated that the bulky hydrophilic moieties of sphingolipids shield the big hydrophobic portion of sterols against the aqueous phase [33]. The interaction of sterols and sphingolipids can lead to formation of membrane domains that withstand treatment with detergents at low temperature; hence, their name detergent-resistant membranes (DRMs) [34,35]. It is becoming clear that different types of DRMs exist [36]. In the context of biological processes where DRMs are postulated to serve as sorting platforms, e.g. for cell surface receptors, the term “raft” is commonly used, but remains ill-defined [37–39].

The internalization step of endocytosis involves the budding of vesicles from the plasma membrane, which are then targeted to and fuse with an early endosomal compartment (Fig. 2). From endosomes, some plasma membrane receptors, SNAREs and lipids are recycled back to their original location. Whether this sorting event requires separate recycling endosomes or occurs in specialized areas of early endosomes is under debate [17,40]. In any event, due to the recycling process, the

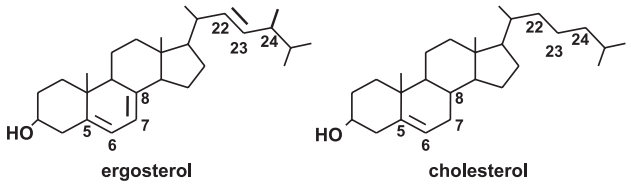

Fig. 1. Structural differences between cholesterol and ergosterol. Compared to cholesterol, the fungal ergosterol contains one more methyl group at position C-24 and two more double bonds at positions C-7 and C-22. The following yeast enzymes are involved in conversions leading to yeast specific sterols: Erg6p adds a methenyl-group at C-24, which is reduced by Erg4p; Erg2p isomerizes a C-8 to a C-7 double bond; Erg5p and Erg3p introduce double bonds at C-22 and C-5, respectively.

steady-state lipid composition of later endocytic compartments shows reduced sterol contents as compared to the plasma membrane [26].

Endocytosed material destined for degradation is routed through multivesicular body (MVB)/late endosomal compartments to the degradative compartments, lysosomes in mammalian cells and vacuoles in yeast (Fig. 2). An unusual glycerophospholipid has been found in late endosomes of mammalian cells. The internal membranes of this late endocytic compartment contain high amounts of lysobisphosphatidic acid (LBPA) [41]. Lysosomal/vacuolar membranes generally contain little sterol [26,27,42].

4. Methods to study the role of sterols in endocytosis

Numerous assays to study endocytosis in mammalian cells and yeast are well established. There are fluorescent compounds whose internalization and transport is followed by microscopy [43–45], endocytosed toxins are detected [46] and radiolabeled ligands or nutrients are quantified upon incorporation [47–49]. All these protocols have proven useful to investigate the function(s) of sterols in endocytosis.

It turned out that not only the amount of free sterols present [50–53] but also the structural properties of sterols [54,55] play a certain role. Mammalian cells can be depleted of or overloaded with cholesterol using cyclodextrins, e.g. methyl-β-cyclodextrin. Alternatively, it is possible to mask and sequester cholesterol with filipin or nystatin. Altering sterol levels of yeast membranes, on the other hand, is more difficult. Yeast cells have a rigid cell wall, which makes the plasma membrane less accessible. Furthermore, they do not take up extracellular sterols under aerobic conditions. Yeast strains carrying the dominant *upc2-1* mutation take up sterols aerobically [56]. Anaerobiosis and genetically altering oxygen metabolism permit sterol uptake in yeast [57], but most likely affect endocytosis independently from sterols.

However, the yeast system is superior for studying the influence of the sterol structure on membrane transport. Yeast mutants unable to form ergosterol, *erg* mutants, are easily accessible and viable as long as only late steps of ergosterol synthesis are affected [58,59]. Different late *erg* mutants accumulate distinct sets of sterols and permit correlation between sterol structures and endocytic phenotypes [54,55].

5. Sterols and the internalization step of endocytosis

Various types of endocytosis operating simultaneously have been characterized for mammalian cells. They are distinguished by the shape, size and composition of the early endocytic structures as well as the molecular requirements [17,46]. Cholesterol is necessary for most internalization routes.

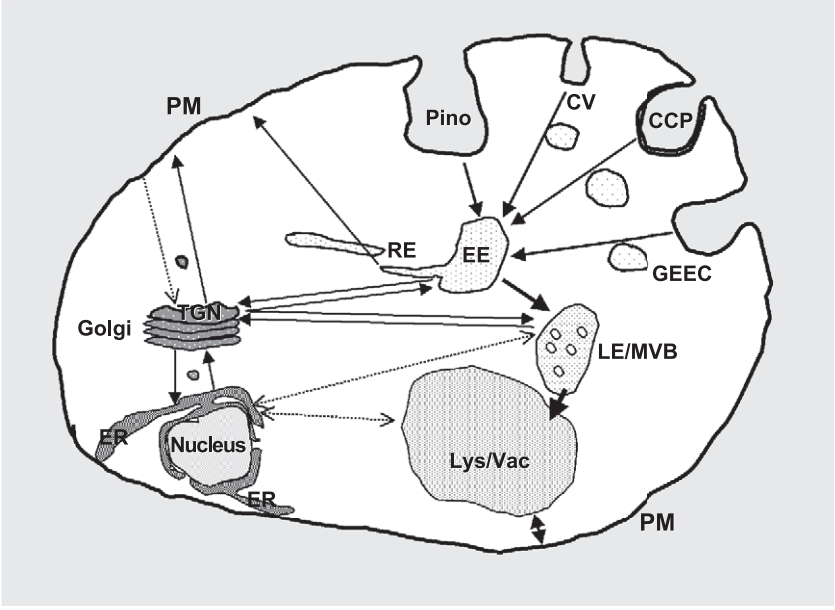

Fig. 2. The endocytic pathway and its intersections with exocytosis. Internalization of plasma membrane (PM) compounds and extracellular material can occur through clathrin coated pits (CCP), caveolae/caveosomes (CV) and macropinocytosis (Pino). GPI-anchored proteins may pass through a distinct early endosomal compartment (GEEC) before reaching early endosomes (EE). Recycling from EE occurs in a specialized domain or separate vesicles termed recycling endosomes (RE). Cargo destined for degradation travels via late endosomes/multivesicular bodies (LE/MVB) to the lysosome/vacuole (Lys/Vac). The secretory pathway endoplasmic reticulum (ER)–Golgi complex–plasma membrane intersects with endocytosis at the trans Golgi network (TGN). Further hypothetical connections are indicated with dotted lines. Structural information on the endocytic compartments was mainly acquired for mammalian cells. Corresponding yeast compartments are morphologically less well characterized due to their limited size.

Caveolae are flask-shaped plasma membrane invaginations implicated in endocytosis of glycosphingolipids, glycosylphosphatidylinositol-anchored proteins, bacterial toxins, non-enveloped viruses and extracellular ligands [60,61]. Caveolins are structurally important proteins for the formation and stability of caveolae and they interact with cholesterol [62]. Depletion or oxidation of cholesterol as well as overexpression of the proteins led to localization of caveolins to intracellular structures, endosomes, Golgi compartment, ER and lipid droplets [63–66]. Upon extraction of cholesterol with methyl-β-cyclodextrin not only caveolae structures disappeared (Fig. 3A), but also endocytosis of transferrin, which is internalized through clathrin coated pits, was strongly reduced in several cell lines (Fig. 3B), but not all [50]. Substantiating that the depletion of cholesterol was responsible for the observed endocytic defects, cyclodextrins incapable of binding cholesterol or cholesterol-loaded methyl-β-cyclodextrin had no influence on transferrin-endocytosis. However, care should be taken when estimating the influence of sterols on the endocytosis of ligand–receptor complexes. Imbalanced sterol situations might, obviously, increase or decrease the affinity in the ligand–receptor interaction as shown for epidermal growth factor receptor. In reality, the number of available receptor binding sites could depend on cholesterol abundance and not the affinity [52].

Internalization via clathrin-coated pits (CCPs) has some similar characteristics as caveolar uptake. Besides cholesterol, dynamin and GTP are required and treatment with methyl-β-cyclodextrin led to severalfold lower transferrin endocytosis and a concomitant accumulation of the receptor at the surface [51]. It was found that cholesterol depletion inhibited CCP budding in Chinese hamster ovary (CHO) cells.

Interestingly, clathrin-independent endocytosis does not require cholesterol [50,67]. Internalization of GPI-anchored diphteria-toxin receptor was insensitive to cholesterol depletion or sequestering, whereas endocytosis of transferrin was perturbed under the same conditions. These results indicated that GPI-anchors do not necessarily direct proteins into cholesterol-rich membrane domains, which can be taken up via clathrin-coated pits or caveolae.

Once more using the methyl-β-cyclodextrin method, it could be shown that cholesterol is necessary for membrane ruffling and actin reorganization [53]. These are prerequisites that pave the way for macropinocytosis. As for caveolar and CCP internalization steps, the role of cholesterol in macropinocytosis might be in forming an appropriate membrane environment. Targeting activated Rac1 to the plasma membrane, which depends on cholesterol, seems to boost formation of phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P_2) in rafts at the plasma membrane. Accumulation of PtdIns(4,5)P_2 precedes membrane ruffling and actin reorganization, required for internalization [53,68–73]. Cholesterol depletion reduced PtdIns(4,5)P_2 synthesis and/or lateral organization in the plasma membrane and might, thereby, cause the endocytic deficiencies observed indirectly [74,75].

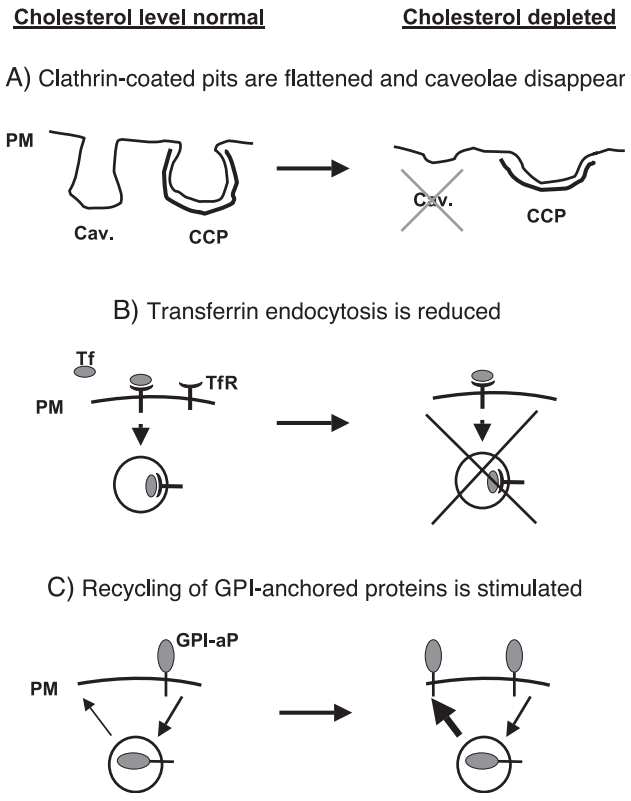

Fig. 3. Cholesterol depletion has diverse effects on the endocytic pathways. Depleting cholesterol, usually by cyclodextrin extraction, alters endocytic structures and the efficiency of certain membrane transport steps. (A) Plasma membrane (PM) structures containing clathrin (CCP) are flattened and caveolae are not recognizable. (B) Transferrin (Tf) and its receptor (TfR) are not endocytosed as under standard conditions. (C) Strikingly, GPI-anchored proteins (GPI-aP) are recycled to the PM at an increased rate.

Cholesterol and its accumulation in plasma membrane raft structures seems to be used by mycobacteria to infect macrophages. Cholesterol is reported to be essential for uptake of mycobacteria by macrophages and also seems to mediate association of TACO, a coat protein that prevents degradation of the prokaryote in lysosomes [76].

Yeast strains unable to synthesize ergosterol, *erg* mutants, accumulate ergosterol precursors in their plasma membranes [58]. It is not sufficiently clear whether the phenotypes seen as a results of the changes in sterols of *erg* mutants are solely due to sterol structural differences or whether *erg* mutants also contain different amounts of total sterols [77,78]. Importantly, a surplus of sterols is most likely stored in the form of steryl esters in lipid particles, which might be considered inert for membrane transport processes [79–82].

Some *erg* mutants, e.g. *erg3, erg4, erg5*, do not have any discernible defects in the internalization step of endocytosis, whereas others, *erg2erg6* and *erg3erg6*, neither accumulate lucifer yellow (LY) in their vacuoles nor internalize alpha-factor at high temperatures [54,55]. Thus, it is not ergosterol that is strictly required for the internalization step of endocytosis, but sterols with sufficient structural similarity.

Most likely, the formation of distinct plasma membrane domains is the key factor for endocytosis of the alpha-factor receptor, Ste2p. Hyperphosphorylation of Ste2p is a prerequisite for ubiquitination, which is necessary for internalization [83]. The same *erg* mutants that are impaired in hyperphosphorylation of Ste2p, *erg2erg6* and *erg3erg6*, do not internalize the receptor (Fig. 4A) [54,55]. The casein kinase I homologues of yeast, Yck1p and Yck2p, are redundant enzymes involved in hyperphosphorylation of Ste2p [55]. Kinase recruitment to the plasma membrane depends on the acyltransferase Akr1p that palmitoylates Yck2p [84–86]. Whether Yck1p and Yck2p directly phosphorylate the alpha-factor receptor is likely, but not yet shown. It remains to be determined whether clustering of Ste2p and/or attachment of kinases to plasma membrane rafts is affected in internalization-incompetent *erg* mutants. Like for cholesterol, the role of ergosterol and structurally similar sterols in early endocytic steps seems to be providing, biophysically speaking, proper membrane structures. It is unlikely that sterols are required for the inward budding of endocytic vesicles at the plasma membrane because they are capable of internalizing FM4-64, a styryl dye that is used as a nonspecific membrane marker of endocytosis.

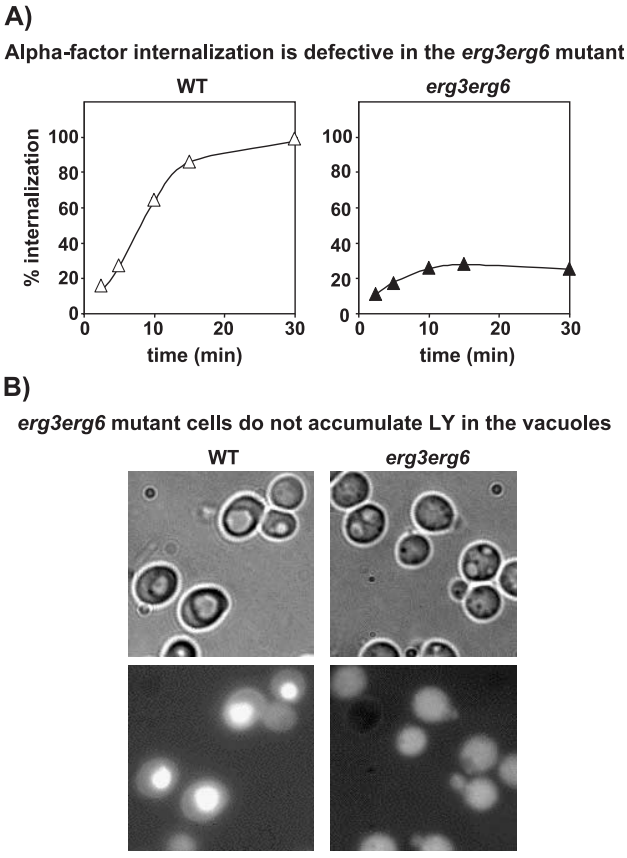

Fig. 4. The importance of the sterol structure. *erg3erg6* mutant cells accumulate sterols structurally different from wild-type sterols. This *erg* mutant strain neither internalizes radiolabeled alpha-factor (A) nor accumulates the fluorescent dye Lucifer yellow (B) in the vacuoles like wild type (WT). (B) Upper panel, differential interference contrast.

Early endocytic transport was recently shown for plant sterols using filipin staining. It turned out that the internalization step in *Arabidopsis thaliana* endocytosis is actin-dependent and, therefore, seems to be related to the mechanism in yeast [87]. Early endosomes contained high levels of sterols, especially after Brefeldin A treatment, which, interestingly, challenged exit of components from early endosomes in plant.

6. Recycling to avoid degradation: where do sterols go?

Once internalized material reaches early endocytic compartments, discrimination is made between components that are destined for degradation in lysosomes or vacuoles and those that are rerouted to the plasma membrane or targetted to the Golgi compartment and ER. Some plasma membrane receptors can release their ligands in acidified endosomes and be recycled [88,89]. Furthermore, SNAREs and lipids that are most abundant in the plasma membrane, sphingolipids and sterols, are sorted for their return to the cell periphery.

At that stage, early endosomal compartments, membrane trafficking gets complicated for several reasons. It is debatable whether separated recycling endosomes exist in mammalian cells or are just specialized domains of early endosomes [17,40]. Furthermore, it has been demonstrated that there is bidirectional transport between endosomes and the trans Golgi network (TGN). Therefore, the virtual borderline between endocytosis and exocytosis gets very faint. Specificity in those transport steps is achieved not only by SNAREs as originally thought [90,91], but also by small GTPases of the Rab/Ypt family. Whereas Rab4 and Rab5 are implicated in early endosome events [92,93] and Rab7 and Rab9 operate in endocytic steps towards late endosomes, lysosomes and Golgi compartment [92,94], Rab11 was found an important role in recycling. Strikingly, transient overexpression of Rab11 led to accumulation of free cholesterol in Rab11-positive organelles that also accumulated transferrin/transferrin receptor complexes. In this intracellular sink, cholesterol was not available for esterification [95]. Recycling endosomal structures are rich in sterols [96–98].

The plant toxin ricin enters into cells by endocytosis, but is ultimately transferred to its site of action, the cytoplasm, from the ER. Only a fraction of internalized ricin is shuttled to the ER from endosomes via the Golgi complex. This transport event is cholesterol-dependent, but glycosphingolipids were not found to be required [99,100]. This is surprising because ricin binds both to glycosphingolipids and glycoproteins [101].

Contradictory results were derived for trafficking of GPI-anchored proteins following internalization [102,103]. It was suggested that a distinct class of clathrin-independent endosomes delivers GPI-anchored proteins directly to the Golgi complex [102]. However, contrasting evidence was presented that GPI-anchored proteins enter recycling endosomes after

passage through a distinct endosome population that receives GPI-anchored proteins in a cdc42-regulated, clathrin-independent process [103]. Strikingly, the recycling of GPI-anchored proteins from endosomes to the plasma membrane seems to be retarded by the presence of normal cholesterol levels, because a sphingolipid analog and transferrin receptors reappeared at the cell surface at least threefold faster than the GPI-anchored proteins [104]. Depletion of cholesterol accelerated GPI-protein recycling when compared to the recycling rate of other components (Fig. 3C). Retention of GPI-proteins in cholesterol-rich domains of recycling endosomes might have important functional consequences.

The recycling pathway is less well defined in yeast, except for the contribution of Rcy1p and the SNAREs Tlg1p and Tlg2p. *rcy1* and *tlg1tlg2* mutant cells are defective for recycling the fluorescent membrane-dye FM4-64 to the plasma membrane when compared to wild-type cells [105]. These cells are also defective in recycling of the SNARE protein, Snc1p [106]. As the sterol profiles of yeast cells match mammalian cells regarding enrichment of sterols along the secretory route and depletion along the endocytic pathway, it can be assumed that sterols are recycled in a similar manner in yeast. An indirect hint in that direction was that certain *erg* mutants, e.g. *erg6*, seem to accumulate more of the membrane marker FM4-64 intracellularly than wild-type cells, especially in peripheral compartments that might be early endosomes [55]. One can speculate that improper sterols are either not recycled efficiently, are trapped in endosomes or at least alter the membrane properties in an unfavorable way for recycling of other compounds.

7. Sterols entering and leaving late endosomes and lysosomes/vacuoles

The degradative part of the endocytic pathway, more specifically, the transition from early to late endosomal compartments, is characterized by the appearance of internal membranes. Thus, these structures are often referred to as multivesicular bodies (MVBs). The term “MVB” is sometimes related to early endosomes or endosomal carrier vesicles, but often used as a synonym for late endosomes [107]. Internal components are destined for degradation that commences in MVBs and proceeds in lysosomes/vacuoles.

There is indication that the unusual glycerophospholipid LBPA is involved in the membrane invagination process to form MVBs [108]. Cholesterol, however, does not seem to be required for reconstituting invagination reactions. On the other hand, annexin II, which was found to distribute between early and late endosomes in a cholesterol-modulated manner, is required for the biogenesis of multivesicular structures destined for late endosomes [109]. By this criterion, cholesterol modulates membrane transport late in the endocytic pathway, most likely by forming sterol-rich platforms in endosomes. In vitro, cholesterol was not required for MVB formation by LBPA, but MVB formation was annexin II-dependent and annexin II localization was modulated by cholesterol.

Tat2p is the high affinity tryptophan permease of yeast [110]. It is transported from the Golgi compartment to the plasma membrane at low tryptophan, and to the vacuole at high tryptophan conditions. The routing decision to these two locations probably occurs in the early endosomes, although the exact mechanism is unknown [111]. Ergosterol and/or sterols with a methyl group in C-24 seem to be required for sorting, because an *erg6* mutant transports Tat2p to the vacuole regardless of whether tryptophan is available. Interestingly, in *erg6* mutant cells Tat2p did not end up in the vacuolar membrane as in ergosterol-synthesizing strains, but entered the MVB pathway and was segregated to the vacuolar lumen. In addition, a GFP–Pep12p construct behaved in a similar manner in *erg6* cells. Genetic and biochemical evidence indicated that poly-ubiquitination of Tat2p and GFP–Pep12p seems to direct those proteins into the MVB pathway in *erg6* cells [111]. As alterations in the sterol structure have strong biophysical implications [25], it can be postulated that the altered properties of sterol-rich domains in early endosomes lead to over-ubiquitination of Tat2p and its missorting to the vacuole lumen in *erg6* mutants.

Assays for fluid-phase endocytosis and membrane marker transport in yeast indicate that sterols have certain function(s) in the late steps of the endocytic pathway. The *erg* mutants *erg2*, *erg2erg3* and *erg3erg6* internalize the fluorescent membrane marker FM4-64 well, but internal transport to the vacuolar membrane is slow and/or incomplete as compared to wild-type. Furthermore, the fluid-phase marker lucifer yellow is not accumulated in the vacuole lumen in those yeast strains (Fig. 4B) [54,55]. The specific role of sterols remains to be determined.

However, it has been claimed that ergosterol is required for the priming step of homotypic vacuole fusion [112]. Vacuolar fragmentation was found for *erg3*, *erg4*, *erg5* and *erg6* mutants. Fragmentation of vacuoles in *erg* mutants seems to depend on the strain background, because in our hands only *erg5* cells show a clear vacuole fragmentation phenotype, but not the others (our own unpublished observation). Addition of cholesterol promoted the in vitro fusion of vacuoles isolated from *erg5* cells, which are otherwise fusion incompetent. Cholesterol and ergosterol seem to stimulate the in vitro fusion of wild-type vacuoles to a similar extent. Consistently, addition of sterol binding drugs nystatin, filipin or amphotericin B inhibited the fusion event [112]. There is further evidence that sterols are involved in vacuole fusion and imbalances in the sterol profiles bring about vacuole fragmentation. Deleting the transcriptional repressor Mot3 and overexpressing the transcriptional activators Upc2 and/or Ecm22 of ergosterol biosynthesis genes led to altered sterol composition and vacuole fragmentation phenotypes [113]. Nevertheless, pleiotropic

effects of deregulating Mot3p, Upc2p and Ecm22p levels cannot be ruled out.

8. Sterols in lipid storage diseases

The involvement of sterols in the vacuole fusion event is surprising as vacuolar as well as lysosomal membranes contain only little sterol [26,27,42]. Considering that mammalian cells can acquire large amounts of intracellular free cholesterol from endocytosis and release of cholesterol from LDL—in late endosomes and lysosomes—extremely efficient cholesterol export routes from these organelles must exist [114]. Cholesterol liberated from LDL is transported to the plasma membrane and the ER. These transport processes can be challenged with progesterone and hydrophobic amines, but it remains unresolved how cholesterol is removed from late endocytic compartments [115]. Some light was shed on that issue by the discovery of the NPC1 protein. Mutations in the *NPC1* gene cause the majority of cases of Niemann–Pick type C-disease, a fatal cholesterol storage disorder that is characterized by accumulation of cholesterol in lysosomes and/or late endosomes [116–118]. The NPC1 protein localizes to a late endosomal compartment and is assumed to participate in sterol efflux from late endosome/lysosomes. It may do so by interacting with NPC2 and MLN64 proteins or, alternatively, these three proteins could have overlapping functions [119]. NPC1 and NPC2 were postulated to regulate cholesterol homeostasis by formation of oxysterols [120]. However, more recent evidence investigating the yeast homologue of NPC1, Ncr1p, indicates that this conserved family of proteins is involved in recycling of sphingolipids from vacuoles/lysosomes and that the observed effects of sterol shuttling might be secondary [121].

Strikingly, not only in Niemann–Pick type C disease but practically in all sphingolipid storage diseases, cholesterol is accumulated in late endosomes and/or lysosomes [122]. It is assumed that the excess sphingolipids in endocytic compartments act as a molecular trap for free cholesterol. Normal human skin fibroblasts treated with naturally occurring and artificial sphingolipids accumulated cholesterol and sphingolipids mainly in late endosomes identified by the presence of LBPA [123].

9. Conclusions

Summing up, there is no clear indication that sterols have a function in endocytosis besides their role in forming membrane domains or contributing in a physical fashion to membrane structure apart from their very strong interaction with caveolin. However, other possible roles do exist; for example, they could be required for the proper membrane insertion, folding or activity of particular membrane proteins. One could also envisage the possibility that such a membrane protein would be active only in a particular compartment due to the sterol composition found there. Unlike sphingoid bases which are required for signaling purposes [124–126] or phosphorylated PtdIns species involved in recruitment of adaptors and membrane sorting processes [127,128], sterols do not have the structural properties to interact with non-membrane proteins. Unless glycosylated sterols [129] have a role in endocytosis, sterols seem to participate in endocytic membrane trafficking as membrane-forming components only.

There is evidence that the interaction of sterols with sphingolipids and the formation of membrane domains is a critical function of the sterol structures [130,131]. Despite the obviously strong affinities between sphingolipids and sterols, most recent data indicate that sterols can be displaced from these domains by ceramides, which might explain the internalization phenomena of cholesterol from plasma membrane upon sphingomyelinase treatment [33,132,133]. However, in addition to the biochemical characterization of detergent-resistant membranes and the biophysical studies to highlight the importance of sterol/sphingolipid-rich domains [134,135], genetic evidence from *S. cerevisiae* demonstrated synergistic effects of the two lipid classes. Affecting biosynthesis of both lipids led to synthetic lethality [78] and mutations in one of the biosynthetic routes changed the structures and/or amounts in the other [136,137].

The importance of specialized membrane domains has mainly been demonstrated at the plasma membrane level. Plasma membrane rafts are proposed to form the platforms for receptor-mediated processes, including signal transduction [138–140], and seem to be the ideal docking sites of proteins that carry acyl- and/or prenyl-anchors, e.g. protein kinases [84–86]. The intracellular functions of membrane domains might be similar, but much more work needs to be done to clarify that point.

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