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Review

Mechanisms of sterol uptake and transport in yeast

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ABSTRACT

Sterols are essential lipid components of eukaryotic membranes. Here we summarize recent advances in understanding how sterols are transported between different membranes. Baker's yeast is a particularly attractive organism to dissect this lipid transport pathway, because cells can synthesize their own major sterol, ergosterol, in the membrane of the endoplasmic reticulum from where it is then transported to the plasma membrane. However, *Saccharomyces cerevisiae* is also a facultative anaerobic organism, which becomes sterol auxotroph in the absence of oxygen. Under these conditions, cells take up sterol from the environment and transport the lipid back into the membrane of the endoplasmic reticulum, where the free sterol becomes esterified and is then stored in lipid droplets. Steryl ester formation is thus a reliable readout to assess the back-transport of exogenously provided sterols from the plasma membrane to the endoplasmic reticulum. Structure/function analysis has revealed that the bulk membrane function of the fungal ergosterol can be provided by structurally related sterols, including the mammalian cholesterol. Foreign sterols, however, are subject to a lipid quality control cycle in which the sterol is reversibly acetylated. Because acetylated sterols are efficiently excreted from cells, the substrate specificity of the deacetylating enzymes determines which sterols are retained. Membrane-bound acetylated sterols are excreted by the secretory pathway, more soluble acetylated sterol derivatives such as the steroid precursor pregnenolone, on the other hand, are excreted by a pathway that is independent of vesicle formation and fusion. Further analysis of this lipid quality control cycle is likely to reveal novel insight into the mechanisms that ensure sterol homeostasis in eukaryotic cells.

Article from a special issue on steroids and microorganisms.

Contents

1. Introduction

Sterols constitute an important class of lipids, which harbor multiple essential functions ranging from signal transduction to protein lipidation. The precise structure and concentration of sterols within eukaryotic membranes affect many membrane associated functions, including vesicle formation and protein sorting, endocytosis, homotypic membrane fusion, the activity of membrane embedded enzymes, the lateral aggregation between proteins and lipids, and the ion permeability of the membrane barrier [1–5]. Due to their concentration-dependent modulation of these functions, the distribution of sterols between different membranes needs to be tightly controlled. The lowest concentration of the free sterol is typically found in the endoplasmic reticulum (ER), where cholesterol levels are maintained at around 5 mol.% of total lipids [6]. The sterol concentration then appears to increase along

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the membranes of the secretory pathway to reach a maximum at the plasma membrane, which harbors 90% of the free sterol pool of the cell and where free sterols account for ~30 mol.% of total lipids [7–12].

Sterols are synthesized and mature in the ER by a cascade of coupled enzymatic reactions. The final product is cholesterol in case of animal cells, stigmasterol, sitosterol, and campesterol in case of plants and ergosterol in fungal cells [13]. While these mature sterols slightly differ in structure between the different kingdoms of life, their basic functions appear to be conserved. It is interesting to note that these kingdom-specific changes in sterol structure are frequently accompanied by corresponding structural changes in the major sphingolipids synthesized, indicating that complex formation between sterols and sphingolipids is one of the conserved properties of these lipids [14–16]. In addition to the free membrane embedded sterol, sterols are also converted to a storage form by esterification with long chain fatty acids. The resulting steryl esters are then stored in lipid droplets from where the free sterol is liberated by the action of specific lipases [17]. Balance between synthesis, transport, esterification of the free sterol and hydrolysis of steryl esters has to be tightly regulated to maintain sterol homeostasis [18]. To prevent the toxic accumulation of free sterols at its site of synthesis, the ER membrane, the free sterol has to be efficiently transported to the plasma membrane [19]. This transport pathway is still incompletely understood but involves both vesicular and non-vesicular components because it is ATP-dependent, but only partially sensitive to brefeldin A, which disrupts vesicular transport at the level of the Golgi apparatus [12,20,21]. The proposition that sterol transport between the ER and the plasma membrane involves both vesicular as well as non-vesicular components is also consistent with the observation that lipid transport continues in yeast mutants that have a conditional block of vesicular transport between the two compartments [22].

In this review, we will summarize the approaches that were taken and the progress made over the past couple of years to understand how sterols are transported between different intracellular membranes, how this lipid transport may be regulated, and how these membranes may establish their characteristic sterol concentration – focusing on studies using yeast as a genetically tractable model organism.

2. Uptake and export of sterols by mammalian cells

Mammalian cells take up cholesterol by receptor-mediated endocytosis of low-density lipoproteins (LDLs) containing cholesteryl esters [23]. LDLs are then delivered to late endosomes or lysosomes where cholesteryl esters are hydrolyzed by an acidic lipase. The liberated free cholesterol is recycled to the plasma membrane or transported to the ER where it is esterified and stored in lipid droplets. The transport of the free sterol from the plasma membrane and the endocytic compartment back to the ER closes the transport cycle between the ER and the plasma membrane and the level of free cholesterol that travels through this route is buffered by the storage and release of sterols from lipid droplets. The back transport of free cholesterol to the ER is inhibited by hydrophobic amines, progesterone, disruption of the cytoskeleton or that of the acidic compartments, but not by ATP depletion, indicating that it occurs through a non-vesicular route [24,25]. This cholesterol transport cycle is defective in certain lipid storage diseases, such as Wolman disease in which a defective acidic lipase results in accumulation of cholesteryl esters in the lysosomal compartment or in Niemann-Pick type C disease where transport of free cholesterol out of the lysosomal compartment is blocked. Functional homologues of these proteins are present in yeast and are implicated in sterol transport in fungal cells as well [3,4,12,17,26,27].

Absorption of nutritional sterols in the intestinal lumen by the enterocytes requires the Niemann-Pick C1 like protein (NPC1L1), a glycosylated brush border membrane protein with 13 predicted transmembrane domains, including a sterol sensing domain [28,29]. Mice lacking NPC1L1 are resistant to diet-induced hypercholesterolemia, suggesting that inhibition of this cholesterol uptake route by the NPC1L1-specific inhibitor, ezetimibe, may provide an effective treatment to reduce LDL-cholesterol levels [30]. Ezetimibe also reduces plasma phytosterol levels in patients with sitosterolemia, indicating that NPC1L1, appears to transport a wide range or sterols. Those foreign sterols then need to be exported again through the action of the heterodimeric ATP-binding cassette transporters (ABC) composed of ABCG5 and ABCG8, mutations in either one of which cause sitosterolemia [31].

Two related ABC transporters, ABCA1 and ABCG1 are required for the major part of cholesterol efflux to serum or high-density lipoprotein (HDL) from macrophage foam cells [32]. Mutations in ABCA1 are associated with Tangier disease, and result in the characteristic severe reduction in HDL levels.

3. Sterol transport in yeast

Yeast is a powerful genetic model organism to study basic cellular processes that are conserved in eukaryotic cells [33]. *Saccharomyces cerevisiae* was adopted early on to study the structure to function relation of different sterols by Bloch and colleagues and subsequently to identify the genes that participate in sterol synthesis and in its regulation [13,34–36]. As in mammalian cells, ergosterol, the mature sterol made by yeast, is synthesized by ER-localized enzymes and is then transported to the plasma membrane, which harbors the highest level of the free ergosterol pool of the cell [9,37]. This transport is fast because newly synthesized ergosterol equilibrates with a half-life of 10–15 min with the plasma membrane-localized sterol pool, which requires that approximately 10^5 ergosterol molecules traffic in and out of the plasma membrane per second, a rate that is 10 times greater than needed to generate a new plasma membrane during cell doubling [22,38]. Equilibration of the newly synthesized ER localized ergosterol pool with that of the plasma membrane is independent of the secretory pathway but requires ATP and a normal level and rate of sphingolipid synthesis [22]. This observation was taken to suggest that concentration of the available free sterol in the ER is comparable to that of the plasma membrane, and that the observed transport reflects the equilibration of this "available" pool of sterols between the two compartments. In this model, the majority of the plasma membrane-localized pool of ergosterol is not freely available because it is trapped into complexes with sphingolipids [39]. Formation of sterol–sphingolipid complexes within the yeast plasma membrane is supported by the observation that the plasma membrane-localized pool of ergosterol in mitotic cells is not accessible to binding to the fluorescent sterol binding polyene filipin [40]. Since various phospholipids also show different affinities for sterols, the intracellular distribution of the free sterol may be based on an equilibration of the concentration of the "active" sterol pool only, that is the fraction of sterols that is not engaged in formation of lipid complexes with either sphingolipids or phospholipids [41]. Such a hypothesis may also explain the wide variation of sterol content between different subcellular membranes [42].

Because sterols are insoluble in the aqueous environment, equilibration of the concentration of free/available sterols between membranes of different organelles requires transfer of the lipid between the two membranes. This lipid transfer can occur through sites of membrane contact between the two organelles, or by transient solubilization of the lipid through its binding to a soluble