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Invited review

Insights into unique physiological features of neutral lipids in Apicomplexa: from storage to potential mediation in parasite metabolic activities

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Abstract

The fast intracellular multiplication of apicomplexan parasites including *Toxoplasma* and *Plasmodium*, requires large amounts of lipids necessary for the membrane biogenesis of new progenies. Hence, the study of lipids is fundamental in order to understand the biology and pathogenesis of these deadly organisms. Much has been reported on the importance of polar lipids, e.g. phospholipids in *Plasmodium*. Comparatively, little attention has been paid to the metabolism of neutral lipids, including sterols, steryl esters and acylglycerols. In eukaryotic cells, free sterols are membrane components whereas steryl esters and acylglycerols are stored in cytosolic lipid inclusions. The first part of this review describes the recent advances in neutral lipid synthesis and storage in *Toxoplasma* and *Plasmodium*. New potential pharmacological targets in the pathways producing neutral lipids are outlined. In addition to lipid bodies, Apicomplexa contain unique secretory organelles involved in parasite invasion named rhoptries. These compartments appear to sequester most of the cholesterol found in the exocytic pathway. The second part of the review focuses on rhoptry cholesterol and its potential roles in the biogenesis, structural organisation and function of these unique organelles among eukaryotes.

Keywords: *Toxoplasma*; *Plasmodium*; Lipid bodies; Rhoptries; Multivesicular bodies; Cholesterol; Cholesteryl esters; Triacylglycerol

1. Apicomplexa and lipid bodies

1.1. Lipid bodies in eukaryotic cells are more than simple energy storage structures

The formation of intracellular lipid particles occurs at some point in the life cycle of nearly all organisms, including plants, mammals, non-mammalian cells, algae and yeast as well as in some prokaryotes (reviewed in Zweytick et al., 2000). These structures are known as lipid bodies, lipid droplets (in adipocytes), adiposomes or oil bodies (in plants). Lipid bodies can be defined as a major form of macromolecular lipid assembly in biological systems. In contrast to the bilayer membrane of organelles, lipid bodies are surrounded by only one monolayer of amphipathic phospholipids, glycolipids and/or sterols that encircles a hydrophobic core of neutral lipids, such as steryl esters, diacylglycerol (DAG) and triacylglycerol (TAG). In mammalian cells, the major enzymes involved in lipid esterification are members of the membrane bound *O*-acyl transferase (MB*O*AT) family (Hofmann, 2000) that include acyl-CoA:cholesterol acyltransferase (ACAT) and acyl-CoA:diacylglycerol acyltransferase (DGAT), producing cholesteryl ester and TAG, respectively (Murphy, 2001).

Two mammalian enzymes ACAT1 and ACAT2, utilising fatty acyl-CoA and cholesterol to produce cholesteryl esters, have been identified (summarised in Buhman et al., 2000; Chang et al., 2001). They are mainly localised within the endoplasmic reticulum, and both proteins display similar enzymologic properties with broad acyl-CoA specificity. The glycerol-3-phosphate pathway, also known

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as the Kennedy pathway is the major route for de novo TAG biosynthesis in all TAG-accumulating organisms (Lehner and Kuksis, 1996). This pathway involves the stepwise acylation of glycerol-3-phosphate and/or dihydroxyacetone phosphate to phosphatidic acid, which in turn is hydrolysed to DAG. DGAT catalyses the final and rate-limiting step, the transfer of the acyl group from acyl-CoA to DAG to form TAG (Lehner and Kuksis, 1996). Two different DGAT have been identified in eukaryotes. DGAT1 is phylogenetically related to ACAT family members (Lehner and Kuksis, 1996; Cases et al., 1998; Oelkers et al., 1998; Bouvier-Nave et al., 2000; Farese et al., 2000; Sorger and Daum, 2002) while DGAT2 is not related to any known enzymes (Cases et al., 2001; Lardizabal et al., 2001). In mammals, DGAT1 is solely a microsomal membrane-protein (Farese et al., 2000) while in plants and yeast, this enzyme has a dual localisation and is found both on the endoplasmic reticulum and lipid (oil) bodies (Bouvier-Nave et al., 2000; Sorger and Daum, 2002). DGAT1 and DGAT2 have similar maximal capacities of TAG synthesis and share, like their ACAT counterparts, broad acyl-CoA specificities (Cases et al., 2001). In addition to the reaction catalysed by DGAT, other mechanisms for TAG synthesis have been observed in plants and yeast (Dahlqvist et al., 2000). Those include an acyl-CoA-independent pathway involving a phospholipid: diacylglycerol acyltransferase, which is distantly related to the mammalian enzyme lecithin:cholesterol acyltransferase (Oelkers et al., 2000). Recently, a new type of DGAT, the bifunctional wax ester synthase/DGAT, has been identified in some bacteria and plants (Kalscheuer and Steinbüchel, 2003).

In multicellular organisms, DGAT displays a variety of physiological functions, including lipoprotein assembly, regulation of plasma TAG concentration or cytosolic DAG levels. As an allosteric activator of the protein kinase C, DAG links extracellular signals to intracellular events, resulting in important biological processes like cell proliferation and differentiation. In addition to its messenger properties, DAG is also a central metabolite in de novo biosynthesis of phospholipids (reviewed in van Blitterswijk and Houssa, 2000). In unicellular microorganisms, TAG can function as a reservoir for metabolic energy and stored TAG may be used as a fatty acid source for phospholipid biosynthesis. Neither neutral lipid biosynthesis nor lipid bodies are essential for yeast growth (Sandager et al., 2002) but accumulation of lipid bodies containing TAG appears to be specifically induced in response to metabolic stress or environmental changes (e.g. osmotic stress, nitrogen depletion; Murphy, 1990, 2001; van Blitterswijk and Houssa, 2000).

There is now increasing evidence that most lipid bodies contain different populations of proteins that are more or less tightly bound to their surfaces. In mammalian cells, proteomic analysis of lipid body-associated proteins identified structural proteins; multiple enzymes involved in the synthesis, storage, utilisation (e.g. perilipin, adipophilin; reviewed in Fujimoto et al., 2004), and degradation of cholesteryl esters and TAG; different Rab GTPases involved in regulating membrane traffic; signaling molecules; and proteins found in caveolae and lipid rafts (Liu et al., 2004; Fujimoto et al., 2004). Higher eukaryotic cells and yeast lipid bodies have strikingly similar proteomes that emphasise common functions in lipid metabolism (Athenstaedt et al., 1999). In plants, the major oil body-associated proteins, oleosins function as stabilisers of TAG. These proteins prevent subcellular oil bodies from coalescence, maintaining them as individual structures in plant seeds even after a long period of storage (reviewed in Zweytick et al., 2000).

It is well known that lipid bodies store neutral lipids utilisable for energy (e.g. fatty acids as respiratory substrates), for membrane biogenesis (e.g. fatty acids, cholesterol) and/or for the formation of specific lipophilic components, (e.g. steroid hormones). However, the constellation of proteins associated with the lipid droplets, as listed above, indicates that these compartments are complex and metabolically active. In fact, they may be a nodal point for multiple intersecting membrane pathways, and be directly involved in membrane lipid recycling. In plants, for instance, another role of oil bodies might be to channel away toxic fatty acids from membranes by incorporating them in TAG (Ohlrogge and Jaworski, 1997). Yeast lipid body particles may serve as a degradation compartment for enzymes that are no longer needed or have been produced in excess (Lum and Wright, 1995).

There are several models describing the biogenesis of intracellular lipid bodies (Fig. 1). First, the ‘budding model’ proposes that droplets of neutral lipids are formed within the bilayer of the endoplasmic reticulum, and then become free cytoplasmic particles, shielded by a phospholipid monolayer after budding of these specific microdomains from the endoplasmic reticulum. The second ‘vesicle flux model’ hypothesises a mechanism of translocation of vesicles from the endoplasmic reticulum, which contain either neutral lipids or proteins, followed by their fusion to produce mature lipid bodies. As a third alternative, the ‘aggregation model’ suggests that specific lipid particle proteins form aggregates in the cytosol and encapsulate neutral lipids that pinched off from the endoplasmic reticulum (summarised in Murphy and Vance, 1999; Zweytick et al., 2000).

Of importance, some of the most widespread human diseases such as atheroma, steatosis, obesity, and certain cancer types (Heid et al., 1998; Murphy and Vance, 1999) are linked to malfunction of lipid body metabolism. In addition, there is a series of recent findings demonstrating a mistargeting of unexpected proteins to the surface of lipid droplets under pathological conditions. Examples include the α-synuclein as a major component of the pathologic lesions characteristic of the Parkinson’s disease (Cole et al., 2002) and the Nir2 protein involved in retinal degeneration (Litvak et al., 2002). This further substantiates the emerging