Comment

Caveolin, Cholesterol, and Lipid Droplets?

Gerrit van Meer

Department of Cell Biology and Histology, Academic Medical Center, University of Amsterdam, 1100 DE Amsterdam, Netherlands

Caveolins constitute the coat of caveolae, specialized domains of the plasma membrane. A large body of evidence suggests that caveolae are enriched in sphingolipids and cholesterol. Besides a role in signal transduction and in the sorting of membrane components, a diverse range of functions has been assigned to the caveolins in the cellular homeostasis of cholesterol. Cholesterol is essential for numerous membrane processes, and its concentration in the cell is governed by a delicate sensor–effector mechanism. However, the basic rules that govern the distribution and dynamics of cholesterol in cells are not understood, a situation that may be changed by an improved insight in the actions of cholesterol-binding proteins like caveolins. Three papers in the present issue report unexpectedly that caveolins can be found on the surface of cytoplasmic lipid droplets (Fujimoto et al., 2001; Ostermeyer et al., 2001; Pol et al., 2001). Lipid droplets consist of apolar lipids and are thought to serve as inert energy storage sites. Caveolin transport to the lipid droplets may represent an “overflow” pathway from the ER under conditions where their concentration in the ER is enhanced (Ostermeyer et al., 2001). More sensational proposals are that caveolin on lipid droplets is a key component in maintaining the cellular cholesterol balance (Pol et al., 2001) and that caveolin populates a raft-like membrane domain on the droplet surface that is involved in intracellular signaling (Fujimoto et al., 2001).

**Cholesterol Homeostasis**

Cells keep their cholesterol content under tight transcriptional control. One essential element of the control machinery is a cholesterol sensor in the ER membrane (Brown and Goldstein, 1997). Apparently, the cholesterol content of the ER accurately reflects the cholesterol status of the cell. This is remarkable because most cellular cholesterol resides in the plasma membrane with very low levels in the ER (Lange et al., 1999). In addition, not all changes in cellular cholesterol occur at the ER. Besides cholesterol synthesis in ER and peroxisomes (Olivier and Krisans, 2000), cholesterol is released from lipoprotein cholesterol esters in late endosomes and lysosomes, and in lipid droplets. Cholesterol is removed from cells by “reverse transport” onto high density lipoprotein at the outside of the plasma membrane, mediated by the ATP-binding cassette transporter A1, ABCA1, and by esterification in the ER membrane by acyl CoA–cholesterol acyltransferases, after which the cholesterol esters are stored in lipid droplets or secreted as lipoproteins. In specialized cells, cholesterol is converted to bile acids or steroid hormones in the mitochondria. The fact that the cellular cholesterol level can be sensed in the ER even though the bulk of the cellular cholesterol is in the plasma membrane and endosomes where an essential part of the cholesterol in- and efflux takes place, raises the question how the cellular cholesterol pools are connected.

As a basic rule, the intracellular disposition of cholesterol is governed by cholesterol’s high affinity for sphingolipids and saturated phospholipids, which are highly enriched in the plasma membrane, the trans-Golgi network and endocytic membranes. The ER contains unsaturated glycerophospholipids, with particularly low affinity for cholesterol. Sphingomyelin and the complex glycosphingolipids are synthesized on the luminal surface of the Golgi complex and cannot spontaneously translocate across the Golgi membrane or the plasma membrane. Thus, sphingolipids are limited to the exo- and endocytic vesicular transport circuits, and virtually absent from mitochondria, peroxisomes, and also from lipid droplets (Zinner and Daum, 1995). The concentration gradient of sphingolipids (and thereby of cholesterol) across the Golgi stack appears to be steep, now that more and more enzymes of sphingolipid biosynthesis have been assigned to the trans-Golgi network. The gradient is maintained by forward transport of the sphingolipids towards the plasma membrane. In our current view, sphingolipids aggregate in the Golgi membrane due to their structural properties. These lateral domains, “afts,” are preferentially excluded from the retrograde pathway, which probably involves both a physical principle, sphingolipid membranes have a lower tendency to bend, and the segregation of membrane-spanning proteins that function in vesiculation.

Cholesterol is essential for the segregation of the sphingolipid domains. By keeping these lipids in a fluid state it allows the coexistence of two fluid domains, a liquid-ordered sphingolipid-cholesterol domain beside a liquid-disordered...
domain of unsaturated glycerophospholipids (Brown and London, 2000). The partitioning of cholesterol with sphingolipids and their anterograde vesicular transport, with a typical half-time of 30 min between ER and plasma membrane, describes only part of cholesterol’s itinerary in the cell. Cholesterol equilibrates across aqueous spaces as a monomer with a half-time of 1–2 h (Phillips et al., 1987). A final transport step that is relevant to understanding the partitioning of cholesterol is its translocation across lipid bilayers. The literature on this is confused with half-times between seconds and hours, but the transbilayer translocation of cholesterol across plasma membranes probably displays a half-time of 1–2 h (Brasaemle et al., 1988).

Cholesterol accumulates in late endosomes in a variety of diseases and experimental conditions (Liscum and Munn, 1999). In all cases, there appears to be an accumulation of lipids that can trap cholesterol (like sphingomyelin, half-time of cholesterol desorption is ~15 h; Phillips et al., 1987), and a defect in vesicle budding from and into late endosomes. The exact order of events is presently unclear, but two proteins involved in cholesterol transport, the Niemann-Pick type C disease protein (Davies et al., 2000) and the Tangier disease ABCA1 protein (Hamon et al., 2000), may be lipid translocators. Proper hydrolysis and removal of lipids, especially sphingolipids like sphingosine, from endosomes may be required for allowing vesicular transport of cholesterol from the endosomes to Golgi and plasma membrane (Fig. 1).

**Caveolins and Cholesterol**

Many experimental studies have suggested a function in cholesterol transport for caveolae and their characteristic protein caveolin. Caveolae have been defined morphologically as flask-shaped invaginations of the plasma membrane, and were found to be involved in endocytosis and transcytosis. In contrast to clathrin-coated pits, where a clathrin lattice is attached to the cytosolic tails of membrane spanning proteins via adaptor proteins, the cytosolic side of caveolae is covered by a protein oligomer that is directly attached to the lipid phase of the membrane. The protein, caveolin of 21–24 kD contains hydrophilic NH2- and COOH-terminal domains that stick into the cytosol, whereas the central domain of 33 hydrophobic amino acids dips into the lipid phase in a hairpin configuration. The family presently consists of caveolins-1 and -3, which display 85% similarity, and caveolin-2, which lacks the NH2 terminus of caveolins-1 and -3, and occurs as α- and β-isofoms. Caveolin-1 is present in numerous cell types, whereas caveolin-3 replaces caveolin-1 in muscle cells. The bulk of these proteins is located in caveolae on the surface, but they are also present on the Golgi membranes. Caveolin-2 is mostly coexpressed with caveolin-1, and the two can form heterooligomers.

The structure of caveolae depends on cholesterol, and caveolins were found to be cholesterol-binding proteins that end up in detergent-resistant membrane remnants, which are generally interpreted as originating from sphingolipid–cholesterol rafts. Typical raft markers like glycosylphosphatidylinositol proteins and glycosphingolipids were found in caveolae under specific conditions. This, and other indirect evidence, has suggested that caveolae are special sphingolipid–cholesterol rafts. In the Golgi membrane, caveolin-1 has been proposed to have a role in lipid and protein sorting to the apical plasma membrane. At the same time, the exciting finding of cytosolic signaling molecules in caveolae has led to a wide interest in caveolae as being cell surface domains where signaling is partially regulated via the physical state of the lipids. Many of the signaling molecules on the cytosolic surface of the plasma membrane carry saturated C14 and C16 acyl chains, whereas prenylated proteins are excluded. This suggests the existence of lipid domains in the cytosolic surface. The triple serine palmitoylation of caveolin-1 is not required for its localization (Dietzen et al., 1995), although it stabilizes the oligomers (Monier et al., 1996). One of the unresolved issues concerning the structure of caveolae is how caveolin on the cytosolic surface recognizes the putative sphingolipid–cholesterol rafts on the exoplasmic surface. Does caveolin recognize a cholesterol-rich cytosolic lipid domain, or could caveolin interact directly with the hydrophobic part of the sphingolipids or cholesterol in the opposed monolayer?
Caveolin has been reported to bind one molecule of cholesterol, and its membrane binding depends on cholesterol (Murata et al., 1995). In addition, caveolin has been found in soluble complexes with cholesterol and a set of chaperones in the cytosol, which might suggest a role for caveolin in intracellular cholesterol transport (Uittenbogaard et al., 1998). The origin of the complexes is unclear. Although newly synthesized caveolin could complex with chaperones cotranslationally and pick up cholesterol in solution, similar to the model cholesterol-acceptor cycloexetrin, this is unlikely because caveolin's presence in complexes required its acylation (Uittenbogaard and Smart, 2000), which is a later membrane-associated event. Alternatively, the binding of acylated caveolin to the ER membrane could be unstable. How these caveolin complexes could obtain cholesterol specifically from the ER and how they would deliver this cholesterol specifically to caveolae in which it would be confined for over an hour (Uittenbogaard and Smart, 2000) remains enigmatic. Finally, a function of caveolins in cholesterol homeostasis is suggested from the presence of sterol-responsive elements in the caveolin-1 and -2 genes (Bist et al., 1997; Fra et al., 2000).

**Lipid Droplets**

Lipid droplets, also termed lipid particles or lipid bodies, are a characteristic feature of adipocytes, where they fill most of the cytoplasm. However, they occur in every mammalian cell. They consist of a core of triglycerides and cholesterol esters. Their surface is covered with proteins of which the major ones are the perilipins in adipocytes and steroidogenic cells, and adipocyte differentiation-related protein (ADRP) also called adipophilin) in preadipocytes and all other cells (Blanchette-Mackie et al., 1995; Servetnick et al., 1995; Brasaemle et al., 1997). These proteins do not contain long hydrophobic stretches, but ADRP is acylated (Heid et al., 1996), which is probably responsible for its droplet association. The closely related TIP47 is a cytosolic protein that binds to the droplets, when these are abundant. Binding of both proteins is hydrophobic in nature as it is resistant to a carbonate wash (Wolins et al., 2001). Perilipins and ADRP cover most of the surface of the droplet and appear to protect the core lipids from hydrolysis by a hormone-sensitive lipase. Upon hormonal stimulation of lipolysis, perilipins and lipase are phosphorylated by protein kinase A, which uncovers the lipids and productively docks the lipase via a receptor, lipotransin (Syu and Saltiel, 1999). Lipid droplets are surrounded by a meshwork of intermediate filaments (vimentin; McGookey and Anderson, 1983), which may serve to prevent contacts with other droplets or other organelles (Murphy and Vance, 1999).

The last steps in the synthesis of the core triacylglycerols and cholesterol esters are catalyzed by related enzymes, acyl CoA–diaeylglycerol acyltransferase and acyl CoA–cholesterol acyltransferase, which spans the ER membrane five or seven times (Lin et al., 1999; Joyce et al., 2000). The most likely mechanism by which triacylglycerols and cholesterol esters end up in lipid particles is that they first accumulate between the leaflets of the ER lipid bilayer (Osterteyer et al., 2001). After reaching a critical size a lipid droplet would bud off the cytosolic side of the ER membrane (Blanchette-Mackie et al., 1995). The hydrophobic core would thus be coated by a monolayer of amphipathic lipids, regular ER membrane lipids oriented with their hydrophobic tails towards the core and their polar moiety towards the cytosol. In this scenario, it is easy to see how ADRP from the ER (Heid et al., 1996) diffuses laterally onto the lipid droplet, while membrane spanning proteins and lumenal ER proteins are excluded (Fig. 2). It is unclear how the cytosolic surface is curved and how droplet fission occurs, but an involvement of ADRP/perilipin seems likely. Overexpression of perilipins resulted in numerous small lipid droplets (Londos et al., 1995). It seems unlikely that ADRP and perilipins form protein complexes in the cytosol that are subsequently filled with lipids similar to lipoprotein assembly in the ER lumen. There, hydrophobic lipids are transported across the aqueous phase by the microsomal triglyceride transfer protein. No such protein has been identified in the cytosol.

In apparent conflict with the budding model (Fig. 2), membrane spanning and lumenal ER proteins have been observed on lipid droplets (Prattes et al., 2000). In addition, some groups have reported the presence of a lipid bilayer around the droplet (Pol et al., 2001). In milk, lipid droplets are surrounded by a bilayer, but this is obtained by budding of the cytosolic droplet through the plasma membrane (Murphy and Vance, 1999). Various interpretations of the data are possible. (a) Some droplets are still physically connected to the ER membrane (Blanchette-Mackie et al., 1995), giving the impression that the ER bilayer covers the droplet. (b) In some EM studies, the core lipids are extracted during the embedding procedure (McGookey and Anderson, 1983), and the surface monolayer may have reoriented into a fragmented bilayer. (c) A lipid bilayer cannot cover the triacylglycerol-cholesterol ester core directly. Lipid particles covered by a lipid monolayer could bud into the ER lumen, but that is not where they are found. Alternatively, the particle could have separated from the lumenal surface of the ER and budded back out enveloping itself in an ER membrane. This model leaves many questions unanswered, notably how does the difference between droplet proteins and ER proteins arise (Zinser and Daum, 1995)? Also, if this model is correct, it is difficult to see how the cytosolic lipase acquires access to the core lipids. (d) Finally, McGookey and Anderson (1983) have reported whorls of layered membranes at specialized sites on the droplet surface, and provided evidence that these were the sites of core lipid hydrolysis by hormone-sensitive lipase (Brasaemle et al., 2000; Morimoto et al., 2000). This hydrolysis results in a surplus of envelope, which may then fold back upon itself.

The hormone-stimulated hydrolysis of cholesterol esters may be the cause of a high concentration of free cholesterol on the droplet surface (McGookey and Anderson, 1983; Prattes et al., 2000). Free cholesterol in droplets was not observed by others (DiAugustine et al., 1973; Zweytick et al., 2000; Pol et al., 2001). The resulting fatty acids instantaneously leave the droplet to equilibrate with the cytosolic fatty acid pool buffered by fatty acid binding proteins. In contrast, it is not clear what happens to the drop-

---

1 **Abbreviation used in this paper:** ADRP, adipocyte differentiation-related protein.
let’s surface components upon hydrolysis of the core lipids. The surface monolayer may shrink into folded bilayered remnants. Indeed, upon lipolysis perilipins have been observed to localize in small punctate structures in the cytosol by immunofluorescence (Souza et al., 1998; Clifford et al., 2000). Probably, the remnants are removed by phospholipases and proteolytic enzymes.

**Caveolins and Lipid Droplets**

A breakthrough in our understanding of caveolins and cholesterol may come from the present reports that caveolins are found on lipid droplets (Fujimoto et al., 2001; Ostermeyer et al., 2001; Pol et al., 2001). Why was this not observed before? Actually, only the β-isofrom of caveolin-2 preferentially locates to the droplets (Fujimoto et al., 2001), whereas caveolin-2α localizes to the Golgi membrane and, in the presence of caveolin-1, to the plasma membrane. Caveolin-1 and -3 are constitutively located at the plasma membrane. Caveolin-2β is an endogenous, NH₂-terminally truncated form of caveolin-2, but engineered truncation mutants of caveolins-1, -2, and -3 display the same behavior (Pol et al., 2001). Full-length caveolins are relocated to droplets when their concentration in the ER is increased by overexpression, by retention at the ER via an ER retrieval signal, or by the inhibition of vesicular transport from the ER by brefeldin A.

How do caveolins reach the droplet surface? First of all, the retention of caveolin-1 by the added retrieval signal indicates that caveolin-1 is inserted into the ER membrane and is normally transported to the plasma membrane by the exocytic pathway (Ostermeyer et al., 2001). The simplest mechanism by which it can move onto the droplet surface is therefore by lateral diffusion through the continuous cytosolic leaflet of the ER membrane (Fig. 2). Interestingly, in the presence of brefeldin A caveolins redistributed to preexisting lipid droplets within 2 h (Fujimoto et al., 2001) or 5 h (Ostermeyer et al., 2001), whereas droplets have a lifetime of over 24 h in the presence of cycloheximide (Pol et al., 2001). This suggests that newly formed droplets fuse to preexisting ones, or that droplets are in continuity with the ER. However, the fact that caveolin-1 was not chased out of the droplets during recovery from brefeldin A (Ostermeyer et al., 2001), suggests that droplets are normally not in continuity with ER. In addition, this demonstrates that lipid droplets are not an intermediary station for caveolins in a rapid transport pathway from the ER to Golgi and plasma membrane.

How are caveolins enriched on the droplet relative to the rest of the ER membrane? (a) Caveolins could have affinity for the droplet cholesterol that is present in the core in the form of cholesterol ester. Although an interesting possibility, reduction of cholesterol esters by 80% did not reduce droplet caveolin (Ostermeyer et al., 2001). Remarkably, also ADRP seems to have a cholesterol binding site (Frolov et al., 2000). (b) Caveolins could recognize a protein on the droplet surface or a protein comprising the structures surrounding the droplet. The latter possibility is supported by the observations that, upon dissolution of the lipid droplet by Triton X-100, the caveolins stuck to...
some fibrous protein right next to the droplet, and that truncated caveolin-3 seemed to move as a wave into a newly forming droplet (Pol et al., 2001). (c) Caveolins and ADRP could be expelled from the ER onto the free droplet surface by transmembrane proteins. (d) Finally, fusion of new lipid droplets to preexisting ones in the cytosol would generate excess membrane relative to volume. This imbalance might be corrected via phospholipid hydrolysis by a phospholipase A_2 and removal of the resulting lysophospholipids and fatty acids, thus concentrating the surface proteins. Apparently, under normal conditions the exocytic route for caveolins from the ER competes efficiently with lipid droplets, and the information for exocytic transport resides in the NH_2-terminal caveolin tail.

Do caveolins fulfill a physiological function in lipid droplets? The presence of high levels of truncated caveolin-3 on lipid bodies led to a dramatic redistribution of the cellular free cholesterol from the plasma membrane to late endosomes (Pol et al., 2001), by a mechanism similar to that of the hydrophobic amine U18666A. As argued above, such accumulation of cholesterol predicts an accumulation of lipids with high affinity for cholesterol and a defect in vesiculation into or out of the endosomes (Fig. 1). The caveolin mutant may sequester a protein component that is essential for one of the endosomal budding events. Alternatively, caveolin on the lipid droplet might induce a signaling cascade with an inhibitory effect on endosomal budding. Interestingly, during lipolysis the caveolin will be in an environment rich in cholesterol and diacylglycerol. Caveolin can activate PMA/diacylglycerol-dependent protein kinases C via its scaffolding domain (Oka et al., 1997; Rybin et al., 1999), which may be part of a lipolysis-regulated signaling loop. The existence of such a mechanism is supported by the finding of two downstream kinases, mitogen-activated protein kinase and mitogen-activated protein kinase and L.L. Rudel. 2000. ACAT1 and ACAT2 membrane topology segregates a steroid residue essential for activity to opposed sides of the endoplasmic reticulum membrane. Mol. Biol. Cell. 11:3675–3687. Lange, Y., J. Ye, M. Rigney, and T.L. Steck. 1999. Regulation of endoplasmic reticulum cholesterol by plasma membrane cholesterol. J. Lipid Res. 40: 2264–2270. Lin, S., D. Cheng, M.S. Liu, J. Chen, and T.Y. Chang. 1999. Human acyl-CoA: cholesterol acyltransferase-1 in the endoplasmic reticulum contains seven transmembrane domains. J. Biol. Chem. 274:23276–23285. Liscum, L., and N.J. Munn. 1999. Intracellular cholesterol transport. Biochim. Biophys. Acta. 1438:19–37. Londos, C., D.L. Brasaeme, J. Gruiia-Gray, D.A. Servetnick, C.J. Schultz, D.M. Levin, and A.R. Kimmel. 1995. Perilipin: unique proteins associated with intracellular neutral lipid droplets in adipocytes and steroidogenic cells. Biochem. Soc. Trans. 23:611–615. McGooyke, D.J., and R.G. Anderson. 1983. Morphological characterization of the cholesteryl ester cycle in cultured mouse macrophage foam cells. J. Cell Biol. 97:1156–1168. Monier, S., R.G. Parson, F. Vogel, J. Beblik, A. Henske, and T.V. Kurzhalia. 1995. VIP21/caveolin, a membrane protein constituent of the caveolar coat, oligomerizes in vivo and in vitro. Mol. Biol. Cell 6:911–927. Monier, S., D.J. Dietzen, W.R. Hastings, D.M. Lublin, and T.V. Kurzhalia. 1996. Oligomerization of VIP21/caveolin in vitro is stabilized by long chain fatty acylation or cholesterol. FEBS Lett. 388:143–149. Morimoto, C., A. Kiyama, K. Kameda, H. Ninomiya, T. Tsujita, and H. Okuda. 2000. Mechanism of the stimulatory action of okadaic acid on lipolysis in rat fat cells. J. Lipid Res. 41:199–204. Murata, M., J. Peränen, R. Schreiner, F. Wieland, T.V. Kurzhalia, and K. Simons. 1995. VIP21/caveolin is a cholesterol-binding protein. Proc. Natl. Acad. Sci. USA 92:10339–10343. Murphy, D.J., and J. Vance. 1999. Mechanisms of lipid-body formation. Trends Biochem. Sci. 24:109–115. Oka, N., M. Yamamoto, C. Schwemke, J. Kawabe, T. Ebina, S. Ohno, J. Couet, M.P. Lisanti, and Y. Ishikawa. 1997. Caveolin interaction with protein kinase C isoenzyme-dependent regulation of kinase activity by the caveolin scaffolding domain peptide. J. Biol. Chem. 272:33416–33421. Oliver, M.L., and S.K. Kriwan. 2000. Peroxiosomal protein targeting and identification of peroxiosomal targeting signals in cholesterol biosynthetic enzymes. Biochim. Biophys. Acta. 1529:89–102. Ostermeyer, A.G., J.M. Paci, Y. Zeng, D.M. Lublin, S. Munro, and D.A. Brown. 2001. Accumulation of caveolin-1 in the endoplasmic reticulum redirects the protein to lipid storage droplets. J. Cell Biol. 152:1071–1078. Phillips, M.C., W.J. Johnson, and G.H. Rothblat. 1987. Mechanism and consequences of cellular cholesterol exchange and transfer. Biochim. Biophys. Acta. 906:223–276.
Pol, A., R. Luetterforst, M. Lindsay, S. Heino, E. Ikonen, and R.G. Parton. 2001. A caveolin dominant-negative mutant associates with lipid bodies on the caveolin cycling pathway and induces intracellular cholesterol imbalance. *J. Cell Biol.* 152:1057–1070.

Prattes, S., G. Horl, A. Hammer, A. Blaschitz, W.F. Graier, W. Sattler, R. Zechner, and E. Steyerer. 2000. Intracellular distribution and mobilization of unesterified cholesterol in adipocytes: triglyceride droplets are surrounded by cholesterol-rich ER-like surface layer structures. *J. Cell Sci.* 113:2977–2989.

Rybin, V.O., X. Xu, and S.F. Steinberg. 1999. Activated protein kinase C isoforms target to cardiomyocyte caveolae: stimulation of local protein phosphorylation. *Circ. Res.* 84:980–988.

Servetnick, D.A., D.L. Brasaemle, J. Gruia-Gray, A.R. Kimmel, J. Wolff, and C. Londos. 1995. Perilipins are associated with cholesteryl ester droplets in steroidogenic adrenal cortical and Leydig cells. *J. Biol. Chem.* 270:16970–16973.

Souza, S.C., L.M. de Vargas, M.T. Yamamoto, P. Lien, M.D. Franciosa, L.G. Moss, and A.S. Greenberg. 1998. Overexpression of perilipin A and B blocks the ability of tumor necrosis factor alpha to increase lipolysis in 3T3-L1 adipocytes. *J. Biol. Chem.* 273:24665–24669.

Syu, L.J., and A.R. Saltiel. 1999. Lipotransin: a novel docking protein for hormone-sensitive lipase. *Mol. Cell.* 4:109–115.

Uittenbogaard, A., Y.S. Ying, and E.J. Smart. 1998. Characterization of a cytosolic heat-shock protein caveolin chaperone complex. Involvement in cholesterol trafficking. *J. Biol. Chem.* 273:6525–6532.

Wolins, N.E., B. Rubin, and D.L. Brasaemle. 2001. TIP47 associates with lipid droplets. *J. Biol. Chem.* 276:5101–5108.

Yu, W., P.T. Bozza, D.M. Tzizik, J.P. Gray, J. Cassara, A.M. Dvorak, and P.F. Weller. 1998. Co-compartmentalization of MAP kinases and cytosolic phospholipase A2 at cytoplasmic arachidonate-rich lipid bodies. *Am. J. Pathol.* 152:759–769.

Yu, W., J. Cassara, and P.F. Weller. 2000. Phosphatidylinositol 3-kinase localizes to cytoplasmic lipid bodies in human polymorphonuclear leukocytes and other myeloid-derived cells. *Blood.* 95:1078–1085.

Zinser, E., and G. Daum. 1995. Isolation and biochemical characterization of organelles from the yeast, *Saccharomyces cerevisiae*. *Yeast.* 11:493–536.

Zweytick, D., K. Athenstaedt, and G. Daum. 2000. Intracellular lipid particles of eukaryotic cells. *Biochim. Biophys. Acta.* 1469:101–120.