# MINIREVIEW

# Neutral Lipid Bodies in Prokaryotes: Recent Insights into Structure, Formation, and Relationship to Eukaryotic Lipid Depots

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Many prokaryotes are able to accumulate large amounts of lipophilic compounds as inclusion bodies in the cytoplasm. Members of most genera synthesize polymeric lipids such as poly(3-hydroxybutyrate) (PHB) or other polyhydroxyalkanoates (PHAs) (125), whereas accumulation of triacylglycerols (TAGs) and wax esters (WEs) in intracellular lipid-bodies is a property of only a few prokaryotes (10). Like the formation of PHAs, TAG and WE biosynthesis is also promoted in response to stress imposed on the cells and during imbalanced growth, for example by nitrogen limitation, if an abundant carbon source is present at the same time. All these lipids act as storage compounds for energy and carbon needed for maintenance of metabolism and synthesis of cellular metabolites during starvation and in particular if growth resumes. Although neutral lipid metabolism in bacteria, especially WE biosynthesis, has attracted increasing biotechnological interest, there has so far been little interest in medical research in the formation of prokaryotic lipid bodies. However, recent findings suggest that lipid body formation and accumulation of TAGs play an important role in the metabolism of the pathogenic bacteria like *Mycobacterium tuberculosis* (37, 52).

In contrast to the restricted occurrence of storage TAGs in prokaryotes, intracellular TAGs are widespread in many eukaryotes (34, 35, 40, 53, 65, 106, 112, 132, 133). In eukaryotes, storage lipids are also deposited as lipid bodies. Their structure and formation have recently been reviewed in detail by several authors, but additional information on prokaryotic lipid-bodies has been only superficial (98, 100, 151). In contrast, intracellular accumulation of WEs as a storage lipid is a great exception in eukaryotes and occurs only in jojoba (*Simmondsia chinensis*) (147), and PHAs are not at all synthesized as a storage compound in eukaryotes.

The number of studies investigating the enzymatic and structural fundamentals of storage lipid metabolism in bacteria has increased drastically in the last years, providing the knowledge that storage lipids in bacteria have a similar important metabolic function like in eukaryotes. This review will focus on prokaryotic neutral lipid bodies in comparison with the currently discussed models for lipid body structure and their formation in eukaryotes and with PHA inclusions in prokaryotes.

## OCCURRENCE AND FUNCTION OF STORAGE LIPIDS IN PROKARYOTES

Accumulation of at least one type of storage lipid can be found in nearly all prokaryotes. Therefore, it is very likely that lipid accumulation is advantageous for survival in natural habitats and that the capability for their synthesis provides a strong advantage during evolution. The few exceptions of prokaryotes lacking the capability to accumulate lipids (for example, lactobacilli, *Enterobacteriaceae*, and methanogenic bacteria) exist in nutrient-rich habitats, in which accumulation of lipids does not provide an advantage.

**Triacylglycerols.** Large amounts of TAGs have been reported mainly in nocardioforms such as *Mycobacterium* sp., *Nocardia* sp., *Rhodococcus* sp., *Micromonospora* sp., *Dietzia* sp., and *Gordonia* sp. and streptomycetes, which accumulate TAG bodies in the cells and the mycelia (2, 5, 10, 17, 62, 105). Furthermore, TAGs are also frequently accumulated in members of the gram-negative genus *Acinetobacter*, though the amounts are small in comparison to the accumulated WEs (91, 122, 123). In general, TAGs are stored in spherical lipid bodies, with quantities and diameters depending on the respective species, growth stage, and cultivation conditions. For example, cells of *Rhodococcus opacus* and *Streptomyces lividans* contain only few TAGs when cultivated in complex media with a high content of carbon and nitrogen; however, the lipid content and the number of TAG bodies increase drastically when the cells were cultivated in mineral salt medium with a low nitrogen-to-carbon ratio, yielding its maximum in the late stationary growth phase (107, 138). At this stage, cells can be almost completely filled with lipid bodies exhibiting diameters ranging from 50 to 400 nm. One interesting example is *R. opacus* PD630, where lipids can reach more than 70% of the total cellular dry weight (5).

**Wax esters.** The first reports on WE biosynthesis in gram-negative bacteria were published more than 30 years ago, mainly involving the genus *Acinetobacter* (44, 45, 50, 122). Meanwhile, accumulation of WEs was also described for *Moraxella*, *Micrococcus*, and *Fundibacter* (21, 23, 119). WE biosynthesis has also been reported in actinomycetes: for example, in *Corynebacterium*, *M. tuberculosis*, and *Nocardia* (16, 113, 139). In *Acinetobacter calcoaceticus*, WEs can reach a fraction of about 25% of the cellular dry weight, indicating that WEs act as main storage compound (44, 138). In general, in *A. calcoaceticus* only one or a few WE bodies can be observed per cell (122, 128, 138). The shape of WE bodies is not restricted to spherical inclusions, and some authors described

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flat, disk-like, or rectangular inclusions when the cells were cultivated on alkanes or alkanols, respectively (64, 123). WEs are not exclusively produced as intracellular WE bodies, because some strains of *Acinetobacter* sp. and the marine bacterium *Fundibacterium jadensis* also produce extracellular WEs from alkanes (21, 41, 91, 123). However, the function of these extracellular WEs and the mechanisms of their export are not known yet.

The main function of TAGs and WEs is to serve as a storage compound for energy and carbon; however, other functions are also considered. Lipid bodies may also act as a deposit for toxic or useless fatty acids during growth on recalcitrant carbon sources, which have to be excluded from the plasma membrane and phospholipid (PL) biosynthesis (9, 11). Furthermore, many TAG-accumulating bacteria are ubiquitous in soil, and in this habitat, water deficiency causing dehydration is a frequent environmental stress. Storage of evaporation-resistant lipids might be a strategy to maintain a basic water supply. Oxidation of the hydrocarbon chains of the lipids under conditions of dehydration would generate considerable amounts of water which could be useful for the cells. In this context, it could be demonstrated that, besides some other effects important during adaption to water stress, a slow degradation of lipid bodies occurred in cells of *R. opacus* PD630 during prolonged dehydration (12). Currently only little knowledge exists on the mobilization of accumulated neutral lipids under conditions permitting growth or under energy and carbon deficiency, and the enzymes involved in degradation and their regulation are unknown. Alvarez et al. (8) demonstrated mobilization of TAGs in *R. opacus* and *Rhodococcus ruber* to occur in the absence of a carbon source and the presence of ammonium. Under these conditions, up to 90% of the accumulated TAGs were used for cell growth and maintenance of metabolism within 120 h.

**Poly(hydroxyalkanoates).** Similar to TAGs and WEs, PHAs represent storage compounds for carbon and energy in members of most eukaryotic genera. Accumulation of PHA was first reported nearly 80 years ago in *Bacillus megaterium* (83). Since then, many reports on the occurrence of PHAs have been published, including reports on members of the halobacteria, whereas PHA could not be detected in lactobacilli, *Enterobacteriaceae*, and methanogens (58, 66, 67, 115). Almost 150 different hydroxyalkanoic acids have now been described as constituents of PHAs (127). Interestingly, new polythioesters, in which the ester bonds between the building blocks were replaced by thioester bonds, were detected recently (89, 90). The terminology for PHAs is based on the chain length of the incorporated hydroxy fatty acids and refers to PHA$_{scl}$ (*s*hort *c*hain *l*ength [C3 to C5]) and PHA$_{mcl}$ (*m*edium *c*hain *l*ength [C6 to C14]). The best-characterized bacterium with respect to PHA metabolism is *Ralstonia eutropha*. Accumulation of PHAs in *R. eutropha* results in the formation of approximately 10 to 20 nearly spherical intracytoplasmic inclusions per cell, with a diameter of up to 500 nm and amounting to up to 90% of the cell dry weight (13). A few actinomycetes, like, for example, *R. ruber*, are capable of simultaneously synthesizing and accumulating similar amounts of TAGs and the copolyester poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) from unrelated carbon sources like glucose (6, 72). Readers more interested in details of PHA metabolism or in the enzymes and genes involved therein should consult one of the recent reviews (114, 129).

## STRUCTURE AND PROPERTIES OF PROKARYOTIC LIPID INCLUSIONS

All storage lipids are deposited as intracytoplasmic inclusions (Fig. 1). The general structure of these inclusions seems to be relatively simple. They consist of a hydrophobic core comprising the respective storage lipid and are surrounded by a layer of amphiphilic compounds comprising various proteins and PLs.

**Triacylglycerol inclusions.** Already in the 1940s to 1960s, droplets or vacuoles had been described in various species of the genus *Mycobacterium* (22, 24, 49, 76). However, although their lipophilic nature was recognized, no further investigations were performed. Droplets were also recognized in *Streptomyces*. However, they were simply referred to as vacuoles or PHA inclusions, without chemical analysis (29, 144). First investigations aimed at revealing the structure of lipid-bodies started only in the 1990s with *Streptomyces* sp. (107). The authors described electron-transparent lipid bodies in the cytoplasm with no further internal structure, which occurred in the late stationary growth phase and filled a substantial fraction of the cells. These lipid bodies showed some tendency of coalescence, and it was assumed that they possess a thin surrounding membrane; having about half the width of the plasma membrane. TAG bodies were first isolated and chemically investigated from *R. opacus* strain PD630 by Alvarez et al. (5), who also observed a unique boundary layer (Fig. 1B). These TAG bodies were mainly composed of TAGs (87%), diacylglycerols (~5%), free fatty acids (~5%), PLs (1.2%), and proteins (0.8%). TAGs from *R. opacus* were mainly composed of hexadecanoic acid (36.4% of total fatty acid content) and octadecenoic acid (19.1%) and with considerable amounts of odd-numbered fatty acid residues like heptadecanoic acid (11.4%) and heptadecenoic acid (10.6%) when cells were grown on gluconic acid, acetic acid, or fructose. These carbon sources have to be degraded to acetyl coenzyme A (acetyl-CoA) before fatty acid de novo synthesis occurs, yielding the fatty acids detected in the TAGs. In carbohydrate-grown cells of *R. opacus*, the stereospecific distribution of the acyl residues on the glycerol backbone is not random. The shorter and saturated fatty acid residues were predominantly esterified to the *sn*-2 hydroxyl group, whereas unsaturated fatty acids were predominantly bound at position *sn*-3 (137). The composition of the TAGs strongly varied when the cells were cultivated on propionic acid, which was activated to propionyl-CoA, resulting in a much higher fraction of odd-numbered fatty acids. Furthermore, TAG formation doesn't only depend on fatty acid de novo synthesis, as was revealed by growth experiments using alkanes and phenylalkanes as carbon sources. In this case, alkanes were directly oxidized and activated to the respective acyl-CoA thioesters and incorporated into TAGs (11).

**Wax ester inclusions.** As was noted previously, three different forms of WE bodies were reported to occur in *Acinetobacter* sp. (i) Spherical inclusions in hexadecane-grown cells of *A. calcoaceticus* HO1-N exhibited average diameters of 200 nm and were first referred to as hydrocarbon inclusions. It was discussed that they could represent an intracellular pool of the unmodified hydrocarbon; however, hexadecane was only a minor component of these inclusions, whereas the WE content was more than 50% of the total lipids (122). Freeze-etching of
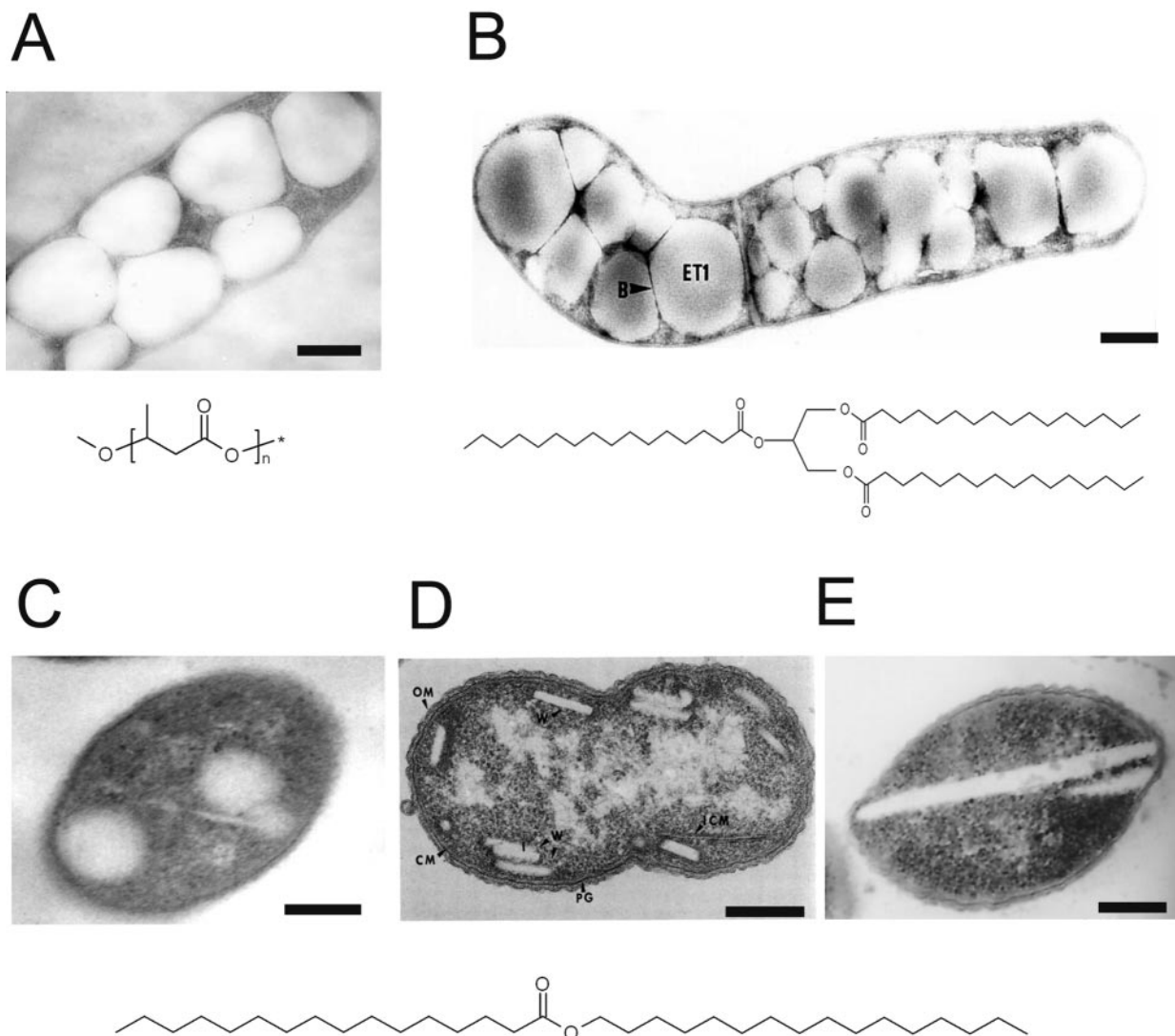
FIG. 1. Intracellular lipid inclusions in prokaryotes and general structures of the lipids. (A) Cell of *R. eutropha* H16 accumulating PHB inclusions (109); (B) cell of *R. opacus* PD630 from late stationary growth phase accumulating large amounts of TAG inclusions (5); (C) cell of *A. calcoaceticus* ADP1 with three spherical WE inclusions; (D) *Acinetobacter* sp. strain HO1-N accumulating small rectangular WE inclusions (123); (E) *Acinetobacter* sp. strain M1 accumulating large, disclike WE inclusions (64). Abbreviations: B, boundary layer; CM, plasma membrane; ET, electron-transparent TAG inclusion; ICM, intracellular plasma membrane; OM, outer membrane; PHA, PHA inclusion; PG, peptidoglycan; W, wax ester inclusion. Bars, 0.2 μm.

these inclusions revealed irregular internal fracture faces, and it was suggested that the inclusions were separated from the cytoplasm by a half-unit membrane. Furthermore, intracellular bilayer membranes were described, which were in direct contact with the plasma membrane. In carbohydrate-grown cells of *A. calcoaceticus* ADP1, WEs also occurred in spherical inclusions with an average diameter of 200 nm, which were also surrounded by PLs (Fig. 1C) (128, 138). Intracellular WEs in strains ADP1 and HO1-N are mainly composed of saturated or monoenic acyl chains with 16 and 18 carbon atoms esterified to fatty alcohols with the same carbon chain length and degree of saturation (71, 123). (ii) Singer et al. (123) reported on electron-transparent, rectangular inclusions with a typical bilayer surface structure in strain HO1-N grown on hexadecanol, measuring 100 to 200 nm in length and 25 to 30 nm in width

(Fig. 1D). The authors described the coformation of intracellular cytoplasmic membranes too. These structures could be isolated, and chemical analysis revealed that they consisted of hexadecylpalmitate (85.6%), hexadecanol (4.8%), and PLs (9.6%). In addition, substantial amounts of lipids were also released into the culture broth. These lipids reached a concentration of 320 μmol per liter and were composed mainly of WEs and TAGs as minor compounds. (iii) In hexadecane-grown cells of *Acinetobacter* sp. strain M-1, WE bodies occurred as electron-transparent, smooth, and disk-like inclusions without a membrane structure (Fig. 1E) (64). These structures grew to about the same diameter as the cell and contained WEs with 32 to 48 total carbon atoms. The authors also performed investigations of the formation of WE bodies in strain M-1 and described the sequential formation of disks, with one

disk completion resulting in the formation of a following one, ending in an accumulation of WE bodies resembling a stack of coins. It is not known whether the different carbon sources or costorage of hydrocarbons or long-chain alcohols caused this morphological divergence observed by the different authors or if it was a specificity of the investigated strains.

**PHA inclusions.** Light microscopic studies identified PHA inclusions as intensively light-scattering granules inside the cytoplasm, and in electron micrographs they appear as spherical and opaque structures with a diameter of up to about 500 nm (Fig. 1A). These polyesters are synthesized by PHA synthase (PhaC), which utilizes CoA thioesters of the respective hydroxy fatty acids as substrates. Under conditions of carbon and energy deficiency, PHA inclusions are mobilized by intracellular PHA depolymerases (PhaZ), which are also localized on PHA inclusions. In *R. eutropha*, three active intracellular depolymerases were detected, but this organism possesses at least five depolymerase genes (110, 120, 149). According to the dimensions of the inclusions and to the molecular weight of one PHB molecule, which may consist of up to approximately 35,000 D-(−)-3-hydroxybutyrate moieties, it was assumed that one average PHB inclusion contains more than 40,000 PHB molecules (126). PHA inclusions appear to have a single boundary layer, which was first believed to be a membrane 15 to 20 nm in thickness or a homogenous layer of PhaC (42, 88, 92). Later reports described the inclusions to be surrounded by a PL monolayer with a 4-nm width and with proteins engaged in PHA synthesis, mobilization, and structural function embedded (38, 61, 93). In *R. eutropha*, four classes of proteins have been demonstrated to occur at the surface of PHA inclusions: PhaC; PhaZ; a transcriptional repressor, PhaR; and phasins (PhaP). PhaP is thought to be a structural protein, because it can be found in large quantities on the inclusions. Recently, it was shown that at least four different PhaPs exist in *R. eutropha*, which are referred to as PhaP1, PhaP2, PhaP3, and PhaP4 (110). In atomic force microscopy, PHA inclusions from *R. eutropha* appear as rough structures with a boundary layer 4 nm in thickness, which is in good agreement with the dimensions of a typical PL monolayer. This layer contained globular, pore-like exaltations 35 nm in diameter, which were believed to be composed mainly of PhaP. These exaltations were connected to each other by linear substructures, resembling some kind of cytoskeleton. After elimination of the boundary layer and the globular structures by treatment with detergents, PHA inclusions showed a smooth, network-like surface of parallel 4-nm arrays, assumed to be the naked PHB core (39).

## PROTEINS ASSOCIATED WITH PROKARYOTIC LIPID INCLUSIONS

Similar to the isolation of eukaryotic oil bodies and prokaryotic PHA inclusions, neutral lipid bodies can be prepared from lysates of the bacterial cells by centrifugation in density gradients (72, 133, 143). Purification of native lipid inclusions is a first crucial step in identifying the structural or enzymatic proteins associated therewith.

**Wax ester bodies.** Scott and Finnerty reported that the protein pattern occurring in purified WE bodies was different from all other membrane fractions isolated from *Acinetobacter* sp. strain HO1-N (122). About 12 proteins were associated with

the inclusions; however, no further characterization of them was done. The first attempts to identify proteins associated with WE bodies were performed with *Acinetobacter* sp. strain 211 (7). These WE bodies contained one major polypeptide of 39 kDa. The first 9 amino acids of the N terminus of this protein were determined, but further characterization was not achieved at that time. Database search revealed that this N terminus shows a high homology to an outer membrane porin from *Acinetobacter baumanii*. Copurification of outer membrane porins as the main proteins of WE bodies occurred as well with inclusions from *A. calcoaceticus* ADP1 (D. Mergelkamp, R. Kalscheuer, and A. Steinbüchel, unpublished results). Recently, it was demonstrated that the wax ester synthase/diacylglycerol acyltransferase (WS/DGAT) is partly located on the surface of WE bodies and also in the cytoplasm and the plasma membrane, as was reported earlier in *A. calcoaceticus* ADP1 (Table 1) (128, 138).

**Triacyglycerol bodies.** Isolated TAG bodies from *R. opacus* and *R. ruber* exhibited a protein pattern strongly resembling the protein pattern of total cell lysates, indicating that a large number of unspecifically bound proteins were copurified (72). This effect is similar to the situation described for oil bodies from plant seeds, which also always contain contaminants of unspecifically bound proteins (95, 133). TAG body-associated proteins were divided into three groups, reflecting their binding properties: (i) probably unspecifically bound proteins by ionic interactions, which could be solubilized by salt treatment or mild detergents; (ii) tightly bound proteins which could be solubilized by treatment with chaotropic reagents; and finally, (iii) extremely tightly bound proteins which even resisted treatment with up to 8% sodium dodecyl sulfate or proteolytic digestion, and which could be only solubilized by sodium dodecyl sulfate plus heat treatment.

Proteins of the latter group were believed to play a role in the structure or formation of TAG bodies and comprised about six proteins in a single bacterium. These proteins were referred to as granule-associated proteins (GAP) and exhibited apparent molecular masses ranging from 15 to 31 kDa. In *R. opacus* PD630, these were GAP15, GAP17, GAP20, GAP26, and GAP31, with the numeration indicating the apparent molecular weight (72). GAP17 from *R. ruber* and *R. opacus* as well as GAP15 from *R. opacus* showed high homologies to ribosomal L7 proteins from different actinomycetes. L7 proteins are part of the stalk region of 50S ribosomal subunits and exhibit low molecular masses (12 to 13 kDa). These proteins possess hydrophobic domains near their N termini which could interact with the lipid matrix of the lipid bodies. Cloning and characterization of the genes of GAP20 and GAP31 from *R. opacus* PD630 identified these proteins as outer membrane porins, as revealed by sequence homology alignments with an outer membrane porin (MspA) from *M. smegmatis*, thus reminding the situation of WE bodies in *Acinetobacter* sp. (104).

All porin homologous proteins purified from WE and TAG bodies were correctly processed and were devoid of their signal peptides. Obviously, these proteins became artificially bound to the inclusions only during cell disruption. Outer membrane porins of *M. smegmatis* are oligomeric proteins with a goblet-like tertiary structure, exposing a mostly nonpolar surface at the goblet's stem and base (43), which could also deeply inter-

TABLE 1. Lipid body proteins and functions

| Cell type | Protein | Function | Reference(s) |
|---|---|---|---|
| **Plants** | | | |
| Seeds and fruits | Oleosin | Structural, maintenance of integrity | 98, 100 |
| | Steroleosin | Dehydrogenase/reductase function in signal transduction | 85 |
| | Caleosin | Probably involved in assembly of proteins on the lipid body surface | 32, 46, 101 |
| Anther pollen | None | | 98 |
| Anther tapetum | Oleosin-like | Structural | 98 |
| Plastids | Plastid lipid-associated protein; fibrillin | Structural | 40, 75, 97, 136 |
| **Animals** | | | |
| Liver hepatocytes, intestine enterocytes | Apolipoprotein | Structural | 98 |
| Adipose enterocytes | Perilipin | Structural | 34, 151 |
| | ADRP/TIP47 | Role in lipid body formation and differentiation, control of lipolysis | |
| Adrenal, testis, ovary (steroidogenic) | Perilipin/ADRP/TIP47 | Control of lipolysis | 151 |
| Mammary epithelium | ADRP/TIP47 | Control of lipolysis | 151 |
| Others (e.g., leukocytes) | ADRP?/TIP47? | Control of lipolysis | 151 |
| **Microorganisms** | | | |
| Yeast | Squalene epoxidase | Lipid metabolism | 82 |
| | Sterol-$\Delta$24-methyltransferase | | 151 |
| | Oxidosqualene cyclase | | 94 |
| | Long-chain fatty acid ligases | | 151 |
| | Acylglycerol-3-phosphate dehydrogenase | | 151 |
| | Alcohol acetyltransferase | | 135 |
| | TAG lipase | | 15 |
| PHA-accumulating prokaryotes | PHA synthase | PHA biosynthesis | 114, 129 |
| | PHA depolymerases | PHA mobilization | 149 |
| | Phasins | Structural and involved in PHA inclusion formation | 126, 143 |
| | PhaR | Regulation of phasin expression | 109 |
| TAG- and WE-accumulating prokaryotes | WS/DGAT? Specific lipases? | Lipid biosynthesis and mobilization | 128, 138 |

act with the lipid core of the inclusions, thus accounting for their strong binding properties. It should be noted that these porins are the only proteins which remained associated with the inclusions under the conditions used in the respective experiments. Removal of all proteins did not affect the structural integrity of the inclusions, suggesting that bacteria do not need any structural proteins to maintain the intracellular lipid bodies. For this reason, prokaryotic lipid bodies are most likely comparable to oil bodies in yeast or some plants which also completely lack structural proteins (98). However, in contrast to PHA inclusions, the difficulties in preparing native prokaryotic TAG and WE bodies imply that future and detailed investigations need in situ studies to reveal a deeper knowledge about the surface of prokaryotic lipid-bodies and their protein equipment.

**PHA inclusions.** Besides PhaC, PhaZ, and PhaR, the most abundant proteins covering PHA inclusions are low-molecular-mass phasins (14 to 28 kDa) (Table 1) (126). In *R. eutropha*, PhaP1 can contribute up to 5% of the total cellular protein in cells cultivated under storage conditions (143). In combination with a proposed layer of PLs, their major role in PHA metabolism is believed to separate the PHA core from the cytoplasm and by this prevent PHA inclusions from coalescing with each other because their surface is no longer hydrophobic, similar to the oleosins in plants (55, 70, 93). How PhaP1 binds to PHA inclusions is unclear. In the C-terminal region of the phasin protein of *R. ruber*, two short stretches of hydrophobic amino acids were detected. Truncated proteins lacking one or both of these stretches were no longer able to bind to PHA inclusions (108). It may be assumed that PhaP1 possesses similar regions at least in the mature protein. In contrast to oleosins of plant seeds, PhaP1 is directly involved in the regulation of the size, quantity, and formation of PHA inclusions and it was shown that the amount of PhaP1 parellels the amount of PHA in the cells (108, 126, 143, 148). The presence or absence of this protein interferes with the shape and number of PHA inclusions, as revealed in mutants overexpressing or lacking PhaP1 (143). In *R. eutropha*, expression of PhaP1 is regulated by the transcriptional repressor PhaR, which binds upstream of the *phaP1* gene in the

promoter region. Lowering of the PhaR titer under conditions permissive for PHA synthesis is mediated by binding of PhaR to the PHA inclusions, thus allowing synthesis of PhaP1 (109). The additional phasins, for which genes were detected in the genome of *R. eutropha* and which occur in much lower concentrations in the cells, also have the capability to bind to PHA inclusions, but their exact function is not known yet (110).

As noted above, in *R. ruber* significant amounts of TAG and PHA exist simultaneously in inclusions. It was shown that in *R. ruber* phasin was localized on any inclusions (72, 108). This suggests that in *R. ruber* the situation is much more complex and that phasins have also a high affinity for TAG bodies, although the processes for formation of both types of inclusions are rather different (see below).

## FORMATION OF PROKARYOTIC LIPID BODIES

Although bacterial neutral lipid and PHA inclusions and eukaryotic lipid inclusions share many parallels, the mechanisms of their formation are most likely very different.

**Wax ester and triacylglycerol bodies.** In *A. calcoaceticus* ADP1, both WE biosynthesis and TAG biosynthesis are mediated by WS/DGAT (73). This enzyme comprises a putative membrane-spanning region but shows no sequence homology to the DGAT1 and DGAT2 families from eukaryotes or the WE synthase from jojoba (19, 27, 28, 60, 80, 118, 121, 150). Under in vitro conditions, WS/DGAT shows a broad capability of utilizing a large variety of fatty alcohols and even thiols as acceptors of the acyl moieties of various acyl-CoA thioesters (74). The substrate specifities of this acyltransferase are extraordinary broad, and it can be considered as a promiscuous enzyme. The specific activity of WS/DGAT with diacylglycerol as an acyl acceptor was one magnitude lower than with long-chain fatty alcohols, thus resembling the distribution of both lipid types in this bacterium (73). WS/DGAT from *A. calcoaceticus* ADP1 represents the first member of a widespread class of bacterial WE and TAG biosynthesis enzymes, because in all bacteria sequenced so far which are known to accumulate neutral lipids, genes for homologous acyltransferases were detected: e.g., in *M. tuberculosis* CDC1551, almost 15 homologues are present (37, 73).

In cells of strain ADP1, which reached their maximum lipid content, WS/DGAT was found not only at the surface of WE bodies but also in the cytoplasm and at the plasma membrane (128). In contrast, this WS/DGAT is almost exclusively localized at the plasma membrane in cells just beginning to store lipids (138). Corresponding to the cellular localization of WS/DGAT, WE formation starts at special domains at the plasma membrane (Fig. 2) (138).

Thus, similar to the formation of eukaryotic lipid bodies at the endoplasmic reticulum (ER) membrane, bacterial neutral lipid synthesis is strictly associated with the plasma membrane, with one all-dominant difference: bacterial lipids are not synthesized between the leaflets of a PL bilayer. In bacteria, WE and TAG formation starts with the docking of WS/DGAT to the plasma membrane, and small lipid droplets (SLDs) are formed. These SLDs are only some nanometers in diameter and remain associated with the membrane-docked WS/DGAT. In this phase of lipid accumulation, SLDs form an emulsive, oleogenous layer at the plasma membrane (Fig. 2A). During prolonged lipid synthesis, SLDs leave the membrane-associated acyltransferase and conglomerate to membrane-bound lipid prebodies (Fig. 2B and C). These lipid prebodies reach distinct sizes, e.g., 200 nm in *A. calcoaceticus* and 300 nm in *R. opacus*, before they lose contact with the membrane and are released into the cytoplasm (138). Free and membrane-bound lipid prebodies correspond to the lipid domains occurring in the cytoplasm and at the cell wall, as recently observed in *M. smegmatis* during fluorescence microscopy and also confirmed in *R. opacus* PD630 and *A. calcoaceticus* ADP1 (33, 138). Inside the lipid prebodies, SLDs coalesce with each other to form the homogenous lipid core found in mature lipid bodies, which appear opaque in electron microscopy, whereas lipid prebodies exhibit a granulous internal structure (Fig. 2D) (138). Although it was not shown in detail, it is very likely that PLs associate in an early stage with membrane-bound SLDs, because it was demonstrated that mature cytoplasmic lipid bodies are at least partially covered by PLs. This hypothesis (Fig. 2E) is also confirmed by experiments in artificial systems, demonstrating the formation of SLDs and lipid prebodies and their final release from solid-phase-supported membranes by scanning force microscopy and quartz crystal microbalance measurements (138).

However, WS/DGAT activity was demonstrated as well on isolated lipid bodies in vitro and WS/DGAT proteins could be localized on cytoplasmic lipid bodies by cytoimmunological methods (128). This may indicate that lipid biosynthesis also occurs on intracellular lipid bodies in vivo. Despite this, it is questionable if the substrates for TAG and WE biosynthesis can reach the lipid bodies already released into the cytoplasm to a significant extent, because most enzymes for biosynthesis of the precursors are typical membrane proteins (36, 116, 145). Furthermore, there is no explanation of how PLs could reach lipid bodies growing in the cytoplasm, which had already lost contact with the plasma membrane. Thus, although bacterial lipid bodies are quite different in many aspects from eukaryotic lipid bodies, they probably also obey the eukaryotic dogma, which means that lipid biosynthesis does not occur on lipid bodies per definitionem (100).

**Poly(hydroxyalkanoate) inclusions.** Although the enzymatic and molecular fundamentals of PHA metabolism have been intensively investigated, our knowledge about formation of PHA inclusions and mobilization of the accumulated PHA is still very scarce. However, the formation of PHAs has to start from soluble substrates which are finally found in insoluble inclusions. Two different models have been proposed for the formation of PHA inclusions (70, 129).

**The micelle model.** The micelle model (Fig. 3A) involves the formation of micelle-like structures from primed PhaC, which itself is a cytoplasmic and hydrophilic enzyme. There is obviously no region of the enzyme protein which mediates binding to hydrophobic surfaces like the PHA inclusions or the plasma membrane. The conversion to an amphiphilic enzyme-product complex is mediated by prolonged synthesis of the hydrophobic PHA molecule. This amphiphilic enzyme-product complex could form micelle-like structures with the growing PHA molecules representing the hydrophobic core and PhaC itself representing the head group. In this model, PhaC remains at the surface of the growing inclusion and is bound covalently to the growing PHA molecule therein. With increasing volume of the inclusion due to increased chain length of the PHA molecules,
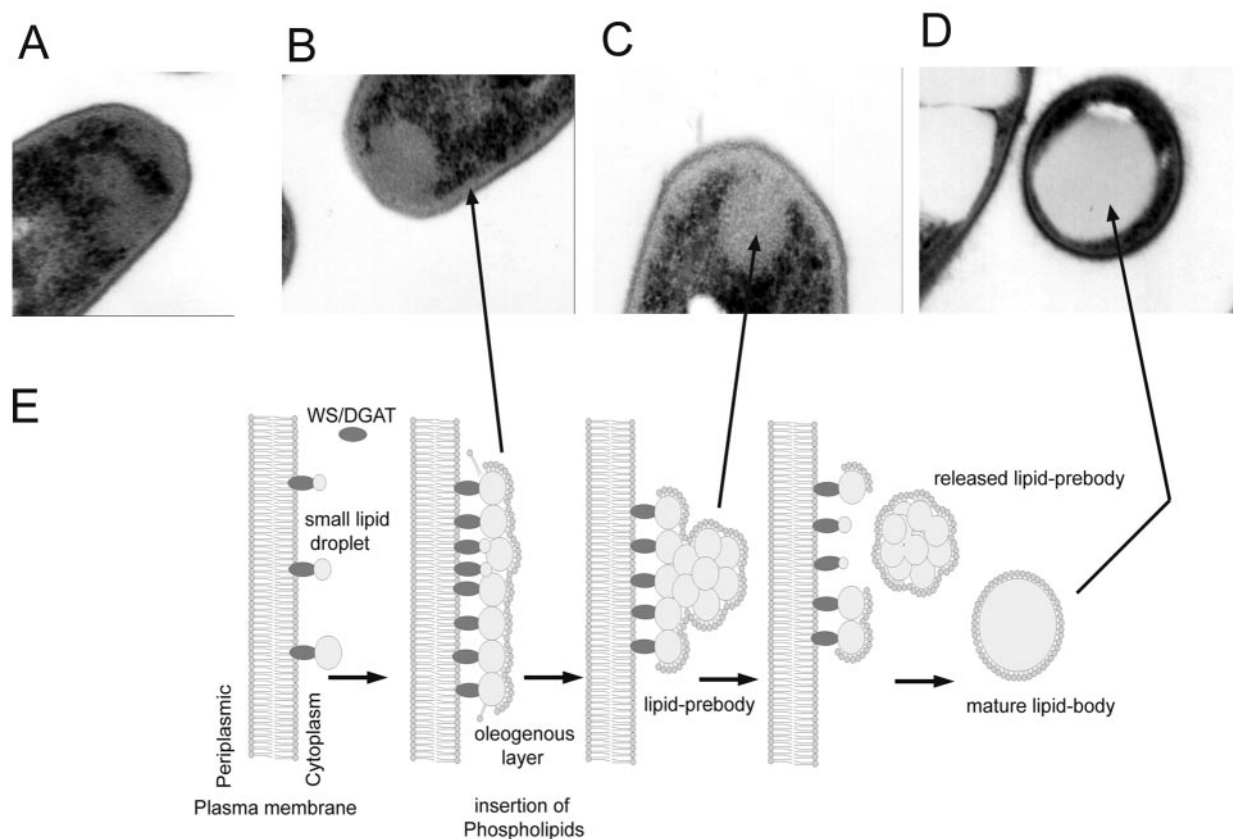
FIG. 2. Stages of the formation of TAG bodies in *R. opacus* PD630 and a generalized hypothetical model for the formation of neutral lipid bodies in bacteria. (A) Formation of an oleogenous layer from SLDs at the plasma membrane; (B) lipid prebody formation through conglomeration of SLDs; (C) partly released lipid prebody; (D) mature, cytoplasm-localized lipid body; (E) scheme (138).

the PhaC proteins are separated and dispersed on the surface. Therefore, an increasing fraction of the hydrophobic PHA core would be directly exposed to the cytoplasm, if these regions are not covered by other amphiphilic molecules. This is obviously done by phasins and PLs, which occupy the empty spaces at the surface and prevent the inclusions from coalescing with each other. Although this model is supported by the strict regulation of phasin expression and analysis of the composition of PHA inclusions, there is no explanation of how PLs are inserted and whether they are required at all. In principle, phasins alone may be sufficient to provide the amphiphilic layer at the surface of the inclusions. It is worth mentioning that PLs had so far only been detected in isolated PHA inclusions; there are no reports on in situ studies actually demonstrating the presence of PLs in the inclusions as they occur in the cells.

**The budding membrane model.** The budding membrane model (Fig. 3B) is partially analogous to the model of lipid body formation in plant seeds. It depends on attachment of PhaC to the plasma membrane. Membrane-bound PhaC could then synthesize a growing PHA molecule into the hydrophobic core between the PL bilayer, thus causing a swelling of the plasma membrane. Similar to the formation of TAGs within the ER membrane in seeds, budding of a PHA inclusion surrounded by a PL monolayer derived from the inner membrane

leaflet, could occur. This model would provide an easy explanation for the incorporation of PLs. In principal, it may be questioned whether or not phasins are at all required for this model or whether PLs would be sufficient for generation of the amphiphilic layer at the surface of the PHA inclusions. This is in contrast to the observation that $PHA_{scl}$ inclusions without phasins were hitherto not observed. Incorporations of phasins will be facilitated if they accumulate at or close to the plasma membrane, from which they are selectively inserted into the nascent PHA inclusion, similar to the proposed targeting of oleosins into seed oil bodies.

## NONPROKARYOTIC LIPID BODIES

To deepen our understanding of bacterial lipid inclusions, it may be helpful to compare their properties with those of eukaryotic lipid bodies, which have been the subject of intensive research in the last decades.

**Plants.** In oleogenous plants like sunflower or rape, the major location of lipid storage is the seed. In oilseeds, lipids are stored in oil bodies with 0.6 to 2.5 μm diameter (133). The predominant storage lipids are TAGs, which can constitute up to 75% of the seed weight (98). Interestingly, seeds of jojoba store lipids as long-chain WEs (147). Similar to prokaryotic lipid bodies, plant oil bodies are composed of a core of neutral
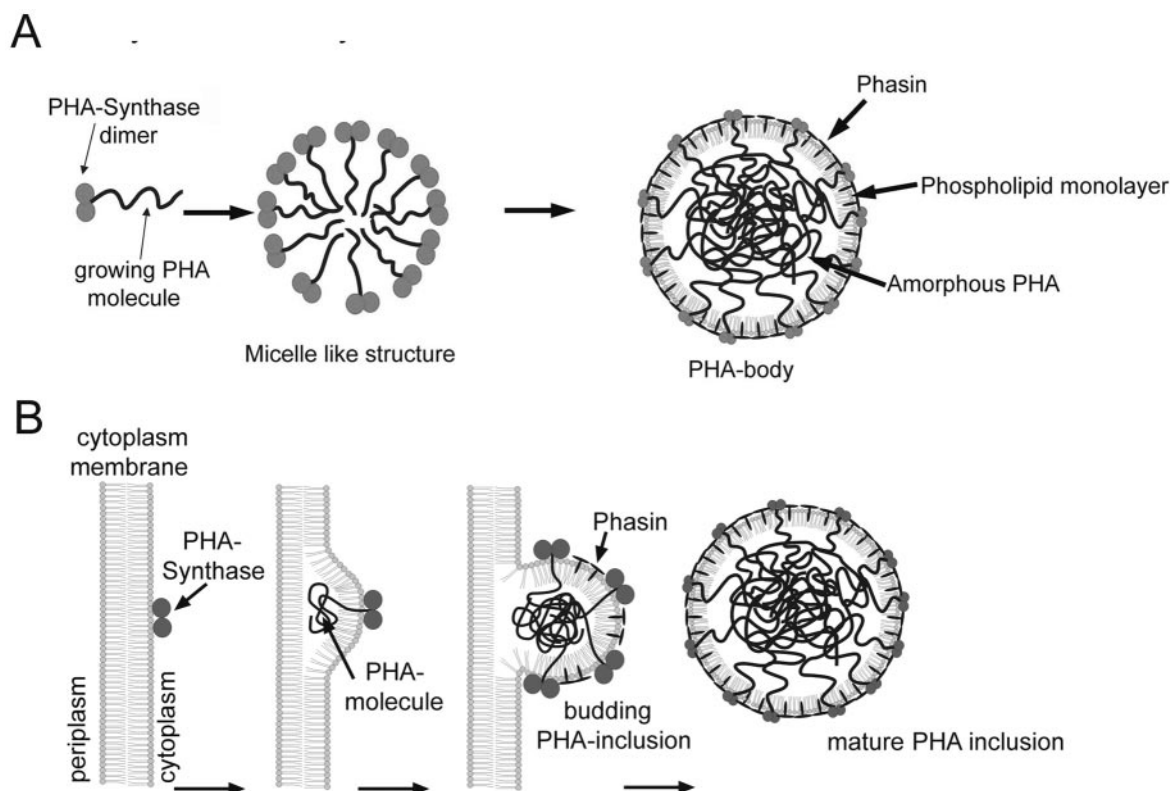
FIG. 3. Suggested models for the formation of PHA inclusions in bacteria. (A) The "micelle" model; (B) the "budding membrane" model (70, 129).

lipids surrounded by a half-unit membrane of PLs (146) and some proteins embedded therein. The amount of total proteins associated with seed oil bodies varies between 0.2% in peanuts and up to 20% in rape (98, 99). Many proteins have been identified which are associated with the oil bodies in plants. This includes caleosins (31, 32, 46, 101), steroleosins (85), and additional proteins of yet unknown function (69). However, the main surface proteins associated with desiccation-tolerant seed oil bodies are oleosins (Table 1) (98). Oleosins are structural proteins of low molecular masses (15 to 26 kDa), depending on the isoforms and species in which they occur. Due to their secondary structure, oleosins form a meshwork-like organization on the lipid body surface. Oleosins are composed of two amphiphilic regions at the N and C termini, whereas the central part represents an expanded conserved domain composed of hydrophobic amino acids, which directly contacts the lipid matrix (30, 78). The secondary structure of this hydrophobic domain was predicted to be an antiparallel β-strain in solvent (63), but more recent experiments performed on native oleosins suggest that it presents mostly as an α-helix (78). A proline knot motif is located at the central loop of this region and was recognized as a putative targeting signal to the TAG core (1). It is assumed that oleosins play a key role in oil body stability in desiccating seeds, since they prevent them from coalescence and attack of unspecific cytosolic lipases or phospholipases (84, 134). Although oleosins play a major role in the structural organization of seed oil bodies, it is improbable that they have an essential function in their formation, because they

are absent from oil bodies of many seeds that do not undergo dehydration and from large oil bodies in the mesocarp tissue of olives and avocados, which are totally devoid of associated proteins (98, 117).

Several different models have been proposed for the formation of seed oil bodies, from which the budding model is the most accepted (Fig. 4). It is based on the formation of small TAG droplets at special subdomains between the two PL leaflets of the ER (48, 103, 140). The intramembranous accumulation of TAGs was confirmed on isolated sunflower microsomes with active TAG biosynthesis, showing that TAG molecules tumble isotropically between both PL leaflets in an environment similar to that in native oil bodies (79). Progressive lipid accumulation will then lead to a swelling of the ER and subsequently to the budding of a lipid body surrounded by a PL monolayer derived from the outer ER leaflet. The coating of nascent oil bodies with oleosins is mediated by a cotranslational insertion of the oleosins into the ER membrane (59, 86). This hypothesis was also confirmed by the localization of TAG biosynthesis enzymes at special subdomains of the ER (26, 77, 79, 118).

**Mammalian lipid bodies.** The storage of TAGs is fundamental in almost any animal. The main components of intracellular lipid bodies in mammals are TAGs and steryl esters. The main location of long-term TAG storage is white adipose tissue, in which only a limited number of lipid bodies per cell with a maximum diameter of 100 nm can be observed (98). In hibernating animals and fetuses and neonates of many animals,
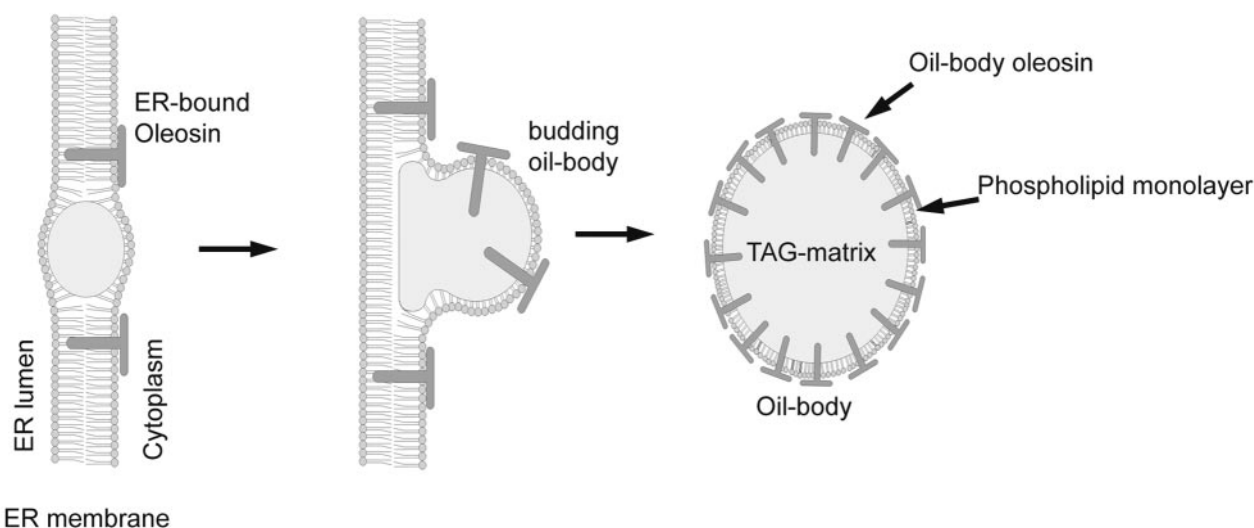
## Oil-body formation in plant seeds



FIG. 4. Model for the formation of lipid bodies in plant seeds. The model involves the accumulation of lipids between both leaflets of the ER membrane. Budding oil bodies are surrounded by a monolayer of phospholipids, which were derived from the outer leaflet of the ER and in which oleosins are embedded (100, 151).

brown adipose tissue also occurs. The quantity of lipid bodies in brown adipocytes is much higher than that in white adipocytes, but their extension is restricted to 2 to 10 nm. In contrast to white adipose tissue, whose main function is TAG storage, the main function of brown adipocytes is thermogenesis through lipolysis and uncoupled respiration. Also in most other cells (for example, in liver, heart, muscle, intestine, and mammary gland), lipid bodies are present (56, 141, 142). Mammalian lipid bodies are spherical structures with a core of neutral lipids coated by a PL monolayer into which specific proteins are embedded. However, only a few of these proteins have been identified, and little is known about their structural properties and functions (Table 1) (34, 151). The main proteins associated with mammalian lipid bodies are proteins of the PAT family, namely *p*erilipins, *a*dipose differentiation-related protein (ADRP) and *t*ail-interacting protein of 47 kDa (TIP47) (96). The best-characterized members of the PAT family are the perilipins, which are highly phosphorylated proteins localized on the surface of lipid bodies of adipocytes and steroidogenic cells and which are involved in lipid body biogenesis and their stabilization. They act as shielding proteins controlling both the storage and lipolysis of TAGs. After phosphorylation by protein kinase A, which is activated by hormonally controlled cyclic AMP, perilipins are released from the lipid bodies, thus enabling hormone-sensitive lipase to bind to the lipid core and to hydrolyze the embedded TAGs. Perilipins are encoded by a single-copy gene in three different forms (A, B, and C) by alternative splicing of the respective mRNA, depending on the tissues in which they occurr. Perilipins A and B from adipocytes have molecular masses of 56 and 46 kDa, both exhibiting a common N-terminal amino acid sequence, which is also very similar to the N termini of ADRP and TIP47 (87). The anchorage of perilipin A is mediated by three hydrophobic regions in the central region of the protein. These regions are flanked by an amphiphilic β-stranded region and the C terminus, which both shield the lipid core from the cytoplasm, and the protein kinase A consensus site (51). In contrast to the restricted occurrence of perilipins in adipocytes and steroidogenic cells, ADRP and TIP47 are expressed in many tissues and cell types. In adipocytes, ADRP seems to play a major role in lipid body differentiation, and during formation of adipocytes from preadipocytes, nascent lipid bodies are surrounded by ADRP (68). The term ADRP is a reference to reflect the early appearance of this protein, which was originally isolated as a strong protein marker in adipocyte differentiation. In a later stage, ADRP is replaced by perilipin as the predominant protein of mammalian lipid bodies (20). ADRP does not exhibit an extended hydrophobic domain like the perilipins; however, a recent study revealed that an amino- and a carboxy-terminal region of ADRP is sufficient for targeting to lipid bodies (102).

Formation of lipid bodies in mammals might occur in a similar way to how it was described for oil body formation in plant seeds; however, the mechanism involved in this process is not well understood. Blanchette-Mackie (18) described a junction between the ER membrane and the lipid body surface, indicating that their formation is associated with the ER. It was discussed whether small lipid bodies in adipocytes undergo coalescence with each other to form one or more large lipid bodies found in matured adipocytes, but the mechanisms involved in controlled coalescence remained unclear (98). This hypothesis was supported by the demonstration that small lipid bodies in adipocytes are associated with cytoskeletal intermediate filaments (e.g., vimentin), which form a cage-like structure around them. This association could prevent fusion and play a role in regulation of controlled coalescence or may play a role in intracellular trafficking of lipid bodies (3, 4, 47).

**Yeast and fungi.** Similar to higher eukaryotes and the pro-
karyotes described above, several yeasts and filamentous fungi,
for example, *Rhodotorula* sp., *Saccharomyces cerevisiae*, *Lipo-
myces starkeyi*, or *Mortierella alpina*, are able to accumulate
large amounts of intracellular lipids after the cells have entered
the stationary growth phase (14, 15, 112, 151). Yeast lipid
bodies share many properties with those from plants and mam-
mals, and their formation is proposed to rely on the same
mechanisms (151). They consist of a hydrophobic core con-
taining TAGs and steryl esters in a similar proportion, sur-
rounded by a PL monolayer accommodating a small amount of
specific proteins. In contrast to plants and mammals, no puta-
tive structural proteins were identified among them, until now.
Similar to their role in higher organisms, yeast lipid bodies
serve as a depot for storage lipids, but it is very likely that they
also play a major role in biosynthesis, intracellular transport,
and channelling of lipid metabolites, because most of the lipid
body proteins identified so far are involved in sterol, fatty acid,
and TAG metabolism (Table 1) (14, 15, 81, 82, 94, 124, 131,
135).

**Plastids.** Lipid bodies in plastids may be similar to prokary-
otic lipid bodies but are only poorly characterized. In chloro-
plasts and chromoplasts, TAGs, sterylesters, and carotenoids
are stored in special structures during growth and under stress
conditions (40, 111, 130, 136). Furthermore, in many fruits, for
example, bell pepper (*Capsicum annuum* L.), chloroplasts dif-
ferentiate while ripening into photosynthetically inactive chro-
moplasts (25). This differentiation involves degradation of
chlorophylls and structural reorganizations, including the deg-
radation of thylakoid membranes and accumulation of lipid
bodies (40). Plastid lipid bodies are termed plastoglobules,
tubules, fibrils, or crystalloids. Their function is not only re-
stricted to lipid storage, but carotenoids also act as attractants
to animals in ripened fruits or as light receptors in photosyn-
thesis. Plastoglobules appear as globular osmiophilic structures
not surrounded by a boundary membrane in electron micros-
copy (54, 57), and it was suggested that they are in direct
contact with the stromal surface of the thylakoid membranes
and the inner envelope of plastids (75). The neutral lipids
inside the plastoglobules form a hydrophobic matrix, which is
surrounded by PLs, glycolipids, and proteins, which are termed
*p*lastid-lipid *a*ssociated *p*roteins (PAPs) or plastoglobulins. It
was suggested that PAPs control carotenoid function on thy-
lakoid membranes and stabilize lipids in plastoglobules, where
they may act as an attachment site to the thylakoid membrane
or as regulatory proteins mediating lipid transfer between the
plastoglobule and the thylakoid membranes (40, 75, 97, 136).

## CONCLUSIONS AND OUTLOOK

The cellular substructures referred to as prokaryotic lipid
bodys or lipid inclusions discussed here share many similar
aspects to lipid bodies in plants, mammals, or yeasts. Both
eukaryotic and prokaryotic neutral lipid bodies are composed
of a hydropobic core of lipids surrounded by a monolayer of
PLs and obviously with no essential need for structural pro-
teins. Formation of eukaryotic and prokaryotic lipid bodies is
directly associated with a cellular membrane, the ER mem-
brane, or the plasma membrane, respectively. Formation of
eukaryotic lipid bodies occurs by lipid accumulation between

the two leaflets of the ER and subsequent budding into the
cytoplasm, whereas in prokaryotes lipid bodies are formed at
the membrane-docked WS/DGAT, leading to the formation of
membrane-associated SLDs and lipid prebodies, which are re-
leased into the cytoplasm after reaching a critical size and from
which the mature lipid bodies are finally formed.

Although we are still at the very beginning of an understand-
ing of the metabolic roles and structures of lipid bodies in
bacteria, several important steps toward progress were recently
made. Nevertheless, many questions remain open. (i) How are
bacterial lipid and lipid body biosynthesis and mobilization
regulated? (ii) Which enzymes catalyze the intracellular mobi-
lization of lipids, and how do they get access to the lipid
bodies? (iii) What is the exact metabolic role of neutral lipid
accumulation in bacteria, especially in pathogens? (iv) How
are PLs exactly inserted into the lipid body surface? (v) How
are the shape and size of the lipid bodies controlled? Similarly,
important aspects of PHA inclusion formation and PHA mo-
bilization remain to be addressed. (i) What are the functions of
the multiple phasin proteins in PHA$_{scl}$-accumulating bacteria?
(ii) How do PHA depolymerases get access to the surface of
PHA inclusions and by this to their substrate during mobiliza-
tion of PHA? (iii) Are PLs present at the surface of the PHA
inclusions, and how are they inserted? To obtain answers, basic
functions of the enzymes and proteins involved in lipid and
PHA metabolism must be investigated. Applications of highly
sophisticated techniques and an interdisciplinary approach of
biochemistry, molecular genetics, cell biology, and biophysics
promise the answers. The knowledge revealed not only will be
important to understand the biogenesis and structure of these
inclusions. It will be also important to understand the metab-
olism of the storage compounds because more and more direct
and indirect evidence for interactions between structure, pro-
teins, and enzymes involved in the biosynthesis or the mobili-
zation of the storage compounds and its regulation is obtained.

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