The Oil Bodies of Liverworts: Unique and Important Organelles in Land Plants

Xiaolan He,1 Yu Sun,1 and Rui-Liang Zhu2

1Botanical Museum, Finnish Museum of Natural History, University of Helsinki, Helsinki, Finland
2Bryology Laboratory, School of Life Science, East China Normal University, Shanghai, China

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Oil bodies of liverworts are intracellular organelles bounded by a single unit membrane containing lipophilic globules suspended in a proteinaceous matrix. They are a prominent and highly distinctive organelle uniquely found in liverworts. Although they have been widely used in taxonomy and chemosystematics, and many of their secondary metabolites are known to be bioactive and are considered as potential sources of medicines, their origin, development and function still remain poorly understood. Recently, biochemical studies have indicated that the isoprenoid biosynthetic pathways in liverworts are similar to those of the seed plants and that oil bodies of Marchantia polymorpha contain a protein complex immunologically related to plastid and cytosolic enzymes of isoprenoid synthesis. Cytoplasmic lipid droplets lacking a binding membrane have recently been recognized as important dynamic organelles playing active roles in cell physiology. Structural proteins, covering the surface of the lipid droplets and preventing them coalescing during desiccation, have been found in seed plants and also in the moss Physcomitrella patens. However, whether liverwort oil bodies play a dynamic role in cell metabolism, in addition to their role as sites of essential oil accumulation and sequestration, has not been formally tested. In this review, we present current knowledge on the oil bodies of liverworts on their origin and development, their role in taxonomy, chemosystematics and potential pharmaceutical applications leading to their functional significance, and we also identify avenues for future studies on this important but long-overlooked organelle.

Keywords oil body, lipophilic globule, liverwort, organelle, lipid droplet, land plant, ontogeny, homology, evolution, ecology, function

I. INTRODUCTION

Extant liverworts include approximately 8000 species and are found on all continents, occupying arid to aquatic niches. Fossil records together with evidence from phylogenetic studies

Address correspondence to Xiaolan He, Botanical Museum, Finnish Museum of Natural History, University of Helsinki, P. O. Box 7, FI-00014 Helsinki, Finland. E-mail: xiao-lan.he@helsinki.fi

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suggest that the first land plants, which evolved approximately 470 million years ago, were liverwort-like (Kenrick and Crane, 1997; Wellman et al., 2003; He-Nygren et al., 2004; Qiu et al., 2006; Rubinstein et al., 2010). These findings indicate that metabolic pathways essential for survival under stress conditions on land must have evolved at a very early stage. It also implies that liverworts might have played an important role in creating a more suitable environment for the subsequent evolution of other land plant groups and that they may help us to understand the vegetative and reproductive changes that favoured the successful radiation of land plants and their adaptations to life in a terrestrial environment.

Progress towards understanding liverwort origins and evolution has been made in resolving phylogenetic relationships of major groups of liverworts and identifying putative key innovations (Davis, 2004; Forrest and Crandall-Stotler, 2004; He-Nygren et al., 2004, 2006; Crandall-Stotler et al., 2005; Heinrichs et al., 2005; He and Glenny, 2010). These studies can well serve as a basis for further investigating adaptive strategies and exploring evolutionary mechanisms and causal factors that have led to extant diversity. One of the putative evolutionary innovations of liverworts is the presence of oil bodies in the cells of both gametophytes and sporophytes. These are intracellular organelles bounded by a single unit membrane containing lipophlic globules (called here as oil body globule, abbreviated as OB globule in the following sections) suspended in a proteinaceous matrix (Pihakaski, 1968; Duckett, 1986). The oil bodies of liverworts exhibit diverse morphological and chemical variations and have been widely used in taxonomy and chemosystematics. Numerous terpenoids as well as aromatic compounds extracted from liverwort oil bodies have been shown to be bioactive and have been suggested as potential sources of medicines (Asakawa, 1995, 2004, 2012). More than 90% of liverwort species contain oil bodies in their cells, and the phylogenetic studies (He-Nygren et al., 2004, 2006) have shown that oil bodies in some groups have been lost independently and most likely at different times during the course of evolution, although they must serve some important function since they have been preserved in most liverworts. However, since the oil bodies have been studied only for a few liverwort species, their origin and development remain poorly understood, and their evolutionary and ecological importance has long been overlooked. Hypotheses on their possible functions, such as, protection against herbivores, pathogens, cold temperatures, UV radiation and desiccation, remain mainly speculative. Recently, biochemical studies have indicated that the isoprenoid biosynthetic pathways in liverworts are similar to those of the seed plants (Nabeta et al., 1994; Tazaki et al., 1995; Adam et al., 1998). Suire et al. (2000) reported that oil bodies of Marchantia polymorpha contain protein complex immunologically related to plastid and cytosolic enzymes of isoprenoid synthesis. However, the mechanisms of metabolic activity in liverwort oil bodies have not been investigated. While the proteins of the liverwort oil bodies are little known, the structural proteins, oleosins, found in cytoplasm lipid droplets of many vascular plant seeds, have recently been reported in the moss Physcomitrella patens (Huang et al., 2009). These proteins cover the surface of the lipid droplets and prevent them coalescing during desiccation. Cytoplasmic lipid droplets lacking a bounding membrane appear to be ubiquitous and are found across kingdoms, although it has been suggested that liverwort oil bodies are fundamentally different in both substructure and development from lipid droplets found in all land plants or algae (Duckett, 1986), further comparative studies, looking at their origin, development, and function, would likely shed light on their evolution in liverworts and also other plant groups. In this paper, we review historical and contemporary studies on the oil bodies of liverworts on their ontogeny, their role in taxonomy, chemosystematics, potential pharmaceutical applications and their functional significance; we also identify avenues for future studies on this unique and important organelle.

II. DISCOVERY OF THE OIL BODY IN LIVERWORTS AND ITS ORIGIN

The oil bodies of liverworts were first described by Hübener (1834) in his description for leafy liverwort Jungermannia taylorii (now as Mylia taylorii) as transparent drops, with shining, membranous texture. Shortly after, Mirbel (1835) observed and illustrated what were called by him as whitish, small, and solid masses or bodies (Figure 1) scattered in the cells of M. polymorpha. Mirbel could not recognize the nature of these bodies, but he suspected they were starch. Gottsché (1843) also called attention on these remarkable structures naming them Zellen Körper (cell bodies) in his study on Haplonitrium hookeri. Von Holle (1857) changed the name to cell vesicles. In 1874, Pfeffer designated them as Ölkörper (oil bodies) mainly on the account of their fatty nature. A genuine membrane surrounding the oil bodies, in which OB globules of various sizes are embedded in a medium of high refractive index, was recognized by some early workers (von Holle, 1857; Pfeffer, 1874; Müller, 1939). Galatis et al. (1978b) suggested that the membrane may have a particular synthesizing activity from the ultrastructure evidence of M. palaeecea. It was suggested that protein might be present in oil bodies (Wakker, 1888; Garjeanne, 1903; Pfeffer, 1904; Pihakaski, 1966, 1968). Pihakaski (1972a, b) confirmed this with light and electron microscope studies, further stating that oil bodies are the main site of lipids in the cells of liverworts. Suire et al. (2000) identified that the oil body is the site of intracellular isoprenoid synthesis in liverworts, supporting the hypothesis that the oil body is an active cell compartment (Galatis et al., 1978b).

Opinions on the origin of liverwort oil bodies from the chloroplast (Wakker, 1888; Kozlowski, 1921), the mitochondrion (Bergdolt, 1926), or the vacuole (Pfeffer, 1874; Garjeanne, 1903; Dombary, 1926; Zirkle, 1932) were presented respectively based on studies at light microscopy level. Later ultrastructural evidence largely supports the hypothesis that oil bodies originate from the dilation of endoplasmic reticulum cisterna...
(Carbonnier and Gavaudan, 1960; Suire, 1970, 2000; Kronest- edt, 1983; Duckett and Renzaglia, 1987; Duckett and Ligrone, 1995; Figure 2). However, hypotheses on the contribution of organelles to oil body formation are substantially different. In her studies of a few leafy liverwort species, Pihakaski (1968) suggested that in *Bazzania trilobata*, oil bodies are formed from vacuole-like structures into which the ER or Golgi apparatus secretes substances as small droplets, whereas in *Lophozia ventricosa* they are developed from fusion of cytoplasmic lipid droplets and the cytoplasm surrounding the OB globules differentiates into the matrix and a limiting membrane of the oil bodies. In contrast, Suire (1970) working with *Radula complanata* concluded that the oil bodies originate from dilated ER cisternae. This conclusion is supported with the study on *Odontoschisma denudatum* and species of *Calypogeia, Diplolophyllum, and Scapania* by Duckett and Ligrone (1995), who rejected the hypothesis of Pihakaski (1966, 1968, 1972a, b) with an argument that the hypothesis of oil body origin from fusion of cytoplasm lipid droplets with vacuole-like structures was probably based on poorly-fixed material. Compared with the leafy liverworts, the origins of the oil bodies in complex thalloid liverworts have been shown to be markedly different. In *M. paleacea* the oil bodies were suggested to originate from
fusion of Golgi-derived vesicles and dilation of the ER membrane (Galatis and Apostolakos 1976; Galatis et al. 1978a, b). Microtubules forming a dense framework around the growing oil bodies were also reported by the authors. Furthermore, the authors underlined that the OB globules are exclusively synthesized within the oil body, while cytoplasmic lipid droplets that are free in the cytoplasm never enter the oil body. This mode of oil body development was also observed in other complex thalloid liverworts *Lunularia cruciata* and *M. polymorpha* by the same authors (Galatis et al., 1978a). However, subsequent studies on complex thalloid *Riella* (Lehmann and Jaster, 1981; Lehmann and Schultz, 1982), simple thalloid *Metzgeria* (Duckett and Renzaglia, 1987), and the re-examination of *L. cruciata* and *M. polymorpha* together with other fourteen species (Suire, 2000), showed no evidence for any contribution of Golgi vesicles and microtubules to oil body development. Suire (2000) concludes that “oil bodies originate from the same cell membrane system and basically develop in the same way in all the Hepaticae,” and considers that *M. paleacea* presents a unique variation in the ontogenic process of the oil bodies.

Until the present day, fine structure investigations on the formation and development of oil bodies have been limited to only a few taxa, nearly all of which have relatively large oil bodies. Methods used for tissue fixation in early studies were usually insufficient and unsatisfactory compared with present day standards. The marked differences in the hypotheses for the formation of the oil bodies seen in previous studies suggest that further studies with a wider range of sampling are required. In particular, the sampling should include species lacking oil bodies such as *Blasia pusilla* and *Anthelia julacea*, and also species having oil bodies that difficult to distinguish from cytoplasmic lipid droplets under a light microscope. Examples are genera *Cephalozi*, *Drucella*, *Kurzia*, and *Metzgeria*. Comparative studies of oil bodies are likely to provide new insights into their origin and development, with comprehensible implications for taxonomy and systematics.

### III. TAXONOMY AND PHYLOGENY

The oil bodies of liverworts occur not only in the gametophytes but also in the sporophytes including seta and spores (Müller, 1939; Suire, 1966, 1970; Pihakasi, 1972a, b). Their size ranges from less than 1 μm to nearly 30 μm in diameter, and they are often the most conspicuous feature of the cell. The oil bodies are best observed and may only be correctly circumscribed from living material. Upon unnaturally fast rates of drying, they disintegrate and eventually become invisible—this may even occur under the strong beam of a light microscope. In natural conditions, they seem to retain their original state even after prolonged periods of drought (Pressel et al., 2009). Seen through a light microscope, oil bodies are usually easily recognizable by their high refractive index and they appear to be colorless, or various densities and tints of brown (such as *Calypogeia* and most Acrobolbaceae), and homogenous or segmented. The oil bodies occur in all mature cells of the gametophyte and sporophyte in the Jungermanniopsida and Haplotrichiales, whereas they usually are restricted to specialized idioblasts in Marchantiopsida and Treubiales. Müller (1939) was the first to suggest a system of classifying the oil bodies into different groups. He recognized nine types of oil bodies according to their appearance, size and form. Schuster (1966) adapted Müller’s classification with some modification and he emphasized that the cells containing a single oil body (called an ocellus) in the complex thalloids except Monoclea, never bear chloroplasts, in contrast to the leafy and simple thalloid group in which chloroplasts are always present in the cells along with the oil bodies. However, ultrastructure evidence (Galatis et al., 1978b, Kronestedt, 1983; Suire, 2000) showed that differentiating ocelli bear chloroplasts like any other cells. Cytoplasmic structures including chloroplasts are pushed to the periphery of the ocellus by the increasing volume of the oil body, which may become difficult to observe under a light microscope. In subsequent taxonomic studies, different schemes of oil body types have been proposed (Gradstein et al., 1977; Schuster, 1992; Kis and Pócs, 1997; Zhu and So, 2001). For example, Gradstein et al. (1977) proposed four types of oil bodies, dividing homogeneous oil bodies into Massula-type and Bazzania-type, and segmented ones into Jungermannia-type and Calypogeia-type (Figure 3). Nevertheless, the shape, number, size, color and distribution of oil bodies vary considerably and they have been recognized as taxonomically important. For example, the oil body types were used as a primary criterion to separate genera of Ptychanthoideae of the family Lejeuneaceae (Schuster and Hattori, 1954). Distinguishing taxa at species and genus levels using oil body characters have also been widely used for leafy and simple thalloid liverworts, especially in families Lejeuneaceae, Frullaniaceae and Radulaceae. For instance, the separation of *Dendrolejeunea* from *Thysananthus* was supported by oil body chemical evidence (Sukkhar et al., 2011). Taxa that lack oil bodies are present in various families, including Blasiaceae, Metzgeriaceae, Cephaloziaeae, Lepidoziaceae, and Antheliales. The cause for the loss of the oil bodies is not known.

So far, taxonomic characterization of oil bodies of liverworts has always been done at light microscopy level; the distinction between an oil body and a cytoplasmic lipid droplet has been questioned when oil bodies are so small that the bounding membrane is difficult to observe. Transitions between homogeneous and segmented or granular types of oil bodies are also present in some families, such as Haplomitriaceae and Lejeuneaceae (Schuster, 1992). Schuster (1992) hypothesized that the plesiomorphic condition in liverworts is that all cells contain a variable number of small oil bodies, similar in size to the chloroplasts or smaller. In a phylogenetic context, the occurrence of unstructured oil bodies in the leafy and simple thalloid groups (such as *Lembidium* of Lepidoziaceae, *Rectolejeunea* of Lejeuneaceae, and *Metzgeria* of Metzgeriaceae), seem to be in a derivative position compared with their allied groups in which structural oil bodies are found (He-Nygrén et al., 2006; Wilson...
OIL BODIES OF LIVERWORTS

FIG. 3. Different types of oil bodies in the cells of liverworts: a–b, Calypogeia-type; c–d, Jungermannia-type; e, Bazzania-type; f, Massula-type—a. Calypogeia azurea, b. Cheilolejeunea anthocarpa, c. Radula constricta, d. Solenostoma truncatum, e. Bazzania tridens, f. Trocholejeunea sandvicensis (color figure available online).

IV. CHEMOSYSTEMATICS AND PHARMACEUTICAL APPLICATION

The chemical nature of oil bodies has been of the interests of many authors as many liverworts present fragrance, especially when crushed. Gottsche (1843) and von Holle (1857) considered that oil bodies contain a fragrant resin or the mixture of etheroil and a resin. Lindberg (1888) and Pfeffer (1874) assumed that they contain fatty oil and protein substance. Lohmann (1903) concluded that the content of oil bodies was terpenes, and Müller (1905) provided evidence that the terpenes were monoterpenes, either sesquiterpenes or the corresponding alcohols. However, the chemical constituents of liverworts were not investigated until 1950s mostly because of the difficulty of obtaining a large quantity of plant material for chemical analysis (Fujita et al., 1956; Huneck, 1967; Huneck and Klein, 1967; Benešova and Herout, 1970; Matsuo et al., 1971 a, b). Isolating pure compounds and determining chemical structures with small amount of material later became possible and easier due to gas chromatography, gas chromatography-mass spectrometry and other analytical methods. So far, about 10% of the more than 700 lipophilic compounds extracted from liverworts have been characterized. Most of the compounds found in liverworts are composed of mono-, sesqui- and diterpenoids and aromatic compounds, such as typical bibenzyls and bisbibenzyls (Asakawa, 2004, 2012). Terpenoids represent the oldest group of small molecular products synthesized by plants. Sesquiterpenoids are the largest class of secondary metabolites in liverworts. Some are also found in vascular plants, while others are unique to liverworts. Diterpenoids are the second largest class of secondary metabolites in liverworts. Pinguisane-type sesquiterpenes and sacculatane-type diterpenoids are only found in liverworts (Figure 4). For example, pinguisane-type sesquiterpenes are significant chemical constituents of Porella species (Asakawa et al., 1981a, b; Asakawa, 1982, 1995, 2001; Hashimoto et al., 2000). Sacculatane diterpenoids have been detected in Pellia, Pallavicinia, Fossombronia, Trichocoleopsis, and Porella (Asakawa, 2004). Many of the sesqui- and diterpenoids are enantiomers of those found in higher plants (Asakawa, 2012). Liverworts have fewer monoterpenes than sesqui- and diterpenoids and according to Asakawa (1995), they...
are mainly responsible for the characteristic odour of liverworts. Together with sesquiterpenes, monoterpenes are the main constituents of essential oils. No terpenoids are found in B. pusilla, which lacks oil bodies (Asakawa, 1988; Wurzel and Becker, 1989). But terpenoids and aromatic compounds have been found in A. julacea, which bears no oil bodies (Asakawa et al., 1980a, b). Unsaturated fatty acids, triglycerides and sterols which are elaborated in high amount in mosses, have also commonly been detected in liverworts. In mosses, terpenoids have rarely been detected.

Terpenoids and lipophilic aromatic compounds have been explored extensively as chemosystematic indicators (Asakawa et al., 1979a, b, c, 1980a, b, 1981a, b; Asakawa, 2004). Asakawa (2004) discussed at length the importance of chemosystematics of liverworts in 36 families at different taxonomic levels. For example, the specific terpenoids and bis-benzyls, such as pinguisane sesquiterpenoids, which are the characteristic markers of the leafy liverworts, are also found in simple thalloid Aneura species (Asakawa, 1995). Asakawa (1995) considers that this indicates the close relationship of the two groups. The sister relationship of the leafy liverworts and the group consisting of Aneuraceae and Metzgeriaceae has been confirmed by recent molecular systematic studies (He-Nygrén et al., 2006). Chemical differences have been used to distinguish family Frullaniaceae from Jubulaceae, and Lepidolaenaceae from Trichocoleaceae (Asakawa, 2004). For a detailed account of the chemosystematics of liverworts, readers are referred to the review paper by Asakawa (2004).

In liverworts, many terpenoids and lipophilic aromatic compounds have been shown to possess remarkable biological activities (Asakawa, 2012). Among others, these include cytotoxicity, anti-HIV inhibitory, antimicrobial, insect antifeedant and mortality, nematocidal activity, anti-obesity, anti-influenza activity, allergenic contact dermatitis, and vasorelaxant effects (Asakawa, 2012). Since M. polymorpha had long been used as traditional medicine to treat liver ailments and to reduce inflammation in China, biological investigation and pharmacological testing have focused on the complex thalloid liverworts. The well-known compounds from Marchantia species, marchantin A-C, biphenyl ethers have been demonstrated to possess significant biological activities, such as antibacterial (Kámory et al., 1995), antifungal (Niu et al., 2006), anti-cytotoxicity (Asakawa et al., 2000) and anti-cancer activity—in the latter case by triggering apoptosis of tumor cells (Shi et al., 2007; Huang et al., 2010). The marchantins have also been revealed to have anti-influenza activity against both influenza A and B viruses (Iwai et al., 2001). Xue et al. (2012) showed that riccardin D, a macroyclic bisbiphenyl isolated from Dumortiera hirsuta, is a novel DNA topo II inhibitor which can induce apoptosis of human leukemia cells. Thus, it has been considered as a potential chemotherapeutic agent for treatment of cancers. Sun et al. (2009) demonstrated that the macroyclic bisbiphenyl dihydroptanchol A (DHA), isolated from Asterella angusta, showed significant multidrug resistance (MDR) reverting activity in chemo-resistant cancer cells. Within the leafy liverworts, Bukvicki et al. (2012) investigated chemical constituents of Porella cordaeana and their results demonstrated that P. cordaeana extracts possess significant antimicrobial potential against food micro-organisms and that high percentages of monoterpenes and sesquiterpene hydrocarbons found in the species could be responsible for the strong antimicrobial activity. Guo et al. (2008) demonstrated that the chemical compounds of Scapania verrucosa have antimicrobial and antitumor activities. Burgess et al. (2000) showed that extracts of Bazzania novae-zelandiae are cytotoxic, with selective activity against human tumor cell lines. Scher et al. (2002) found that Glauceescenolide 1 was the most cytotoxic compound isolated from Schistochila glaucescens against P388 leukemia cells. Nevertheless, liverwort bioactive compounds are increasingly seen as being potentially useful for the production of new drugs. The ability of liverwort oil bodies to undergo dynamic metabolic fluxes of their matrix constituents may provide a competitive evolutionary advantage, as much of the tissue of liverworts is only one cell thick, grows closely to the substrate and has no mechanical protection such as bark or cuticle. This ability may have been a very early prerequisite for survival on land of the earliest land plants.

V. FUNCTION

The possible functions of liverwort oil bodies suggested have been protection from herbivores (Stahl, 1888), pathogens, cold temperatures, excessive light (Hieronymus, 1892), and UV radiation and desiccation (Gavaudan, 1927; Chalaud, 1931). These suggestions made very early on are still been stressed today, although cases that are against these assumptions can all be found. For instance, Anthelia usually grows in sites with intensive light and sometimes in very arid conditions, but it does not bear oil bodies; xeric Riccia species don’t bear oil bodies. Müller (1939) suggested that the oil bodies may have had a physiological function in the past that no longer is expressed today. Pihakaski (1968) assumed that oil bodies have a metabolic function based on their structure and the close connection with the endoplasmic reticulum. As yet effort on testing these assumptions directly or indirectly has been limited. Recently, Pressel et al. (2009) investigated physiological and cytological changes associated with dehydration and rehydration in six liverwort species and they found that the oil bodies of Southbya nigrella retained their original volume during desiccation. According to the authors, the oil bodies collapse during rehydration only to regain their original volume some 48 h after rewetting. It is suggested that the collapse during rehydration indicates a shifting of carbohydrates into the cytosol. Therefore, these authors assume that the oil bodies contain highly concentrated soluble carbohydrate or other moieties whose osmoticum resists water loss and that the oil bodies may have a crucial role in desiccation tolerance.
Fabón et al. (2012) demonstrated that Jungermannia exsertifolia subsp. cordifolia showed a dynamic protection and acclimation capacity to the irradiance level and spectral characteristics of the radiation received by studying the global amount of UV-absorbing compounds (as the bulk UV absorbance of methanolic extracts). Arróniz-Crespo et al. (2004) based on their study of bryophytes collected in Spain, and Otero et al. (2008) based on their Argentina collections, have drawn the same conclusion that the accumulation of UV-absorbing compounds might often increase protection against UV radiation in liverworts, but rarely in mosses. Martínez-Abaigar and Núñez-Olivera (2011) concluded that liverworts seem to have higher amounts of both constitutive and inducible UV-absorbing compounds than mosses. It has been suggested that the accumulation can reduce UV penetration and damage to potential targets (Otero et al., 2008). Thus, the efficient accumulation of UV-absorbing compounds in liverworts could have been one of the factors favoring their success in the colonization of land. UV fluxes were higher during the earlier phases of evolution of land plants, due to the thinner ozone layer (Cockwell and Knowland, 1999), therefore the development of UV-screening compounds might have played a major role in the evolution of plants. However, whether the oil bodies of liverworts have UV-absorbing compounds, has not been investigated.

Whether the oil bodies of liverworts act as an energy storage organelle is not well known. This was discussed in the past but the authors all denied the possibility that the oil bodies could store excessive nutrients. The ground for this was that oil bodies were not detected in the sporophytes (Zwickel, 1932) or that they were found in the cells of sporophytes but there were no chloroplasts in those cells (Pfeffer, 1874; von Küster, 1894; Stewart, 1978). Both oil bodies and photosynthetic activities have been detected in liverwort sporophytes in subsequent studies. Stewart (1978) considered that terpenes, which occur in many plants, do not reserve food.

Cytoplasmic lipid droplets are particularly conspicuous in storage cells in vascular plants. For example, most vascular plant seeds store lipid droplets as a food reserve for germination and post-germinative growth (Huang et al., 2009). Cytoplasmic lipid droplets have recently been recognized as important dynamic organelles playing active roles in cell physiology (Beller et al., 2010; Chapman et al., 2012; Fujimoto and Parton, 2011). However, whether oil bodies of liverworts play a dynamic role in cell metabolism, in addition to their role as sites of essential oil accumulation and sequestration, has not been formally tested. The current hypothesis for lipid droplet biogenesis is that the organelles are derived from the ER. Lipid droplets consist of a hydrophobic core of stored fat, mostly neutral lipids such as triacylglycerols but also sterols and sterol esters, which are surrounded by a protein-bearing phospholipid hemimembrane (Farese and Walther, 2009; Beller et al., 2010; Digel et al., 2010). Recently, major proteins that associate with lipid droplets in some seed plant and algal groups have become known (Murphy, 2001; Abell et al., 2002; Li et al., 2002; Nguyen et al., 2011; Huang et al., 2009; Vieler et al., 2012). Structural proteins, oleosins, have been found in seed plants and also in the moss Physcomitrella patens. These proteins cover the surfaces of lipid droplets and prevent them coalescing during desiccation (Siloto et al., 2006; Shimada et al., 2008; Huang et al., 2009). Oleosins are not present in green algae, although oleosin-like proteins with functions analogous to plant oleosins have been identified (Moellering and Benning, 2010; Peled et al., 2011; Vieler et al., 2012). The proteins of liverwort oil bodies are little known.

VI. FUTURE DIRECTIONS AND PERSPECTIVES

Without doubt, oil bodies are one of most important organelles in liverworts. It is regrettable that while our understanding of lipid droplets in other plant groups, fungi and animals is rapidly evolving, fundamental questions pertaining to the evolution and function of oil bodies in liverworts still remain unanswered. These include: (1) Are the OB globules of the oil bodies of liverworts and the lipid droplets of other plant groups homologous and how are the OB globules and cytoplasmic lipid droplets related within the liverworts? (2) Do oleosin or oleosin-like proteins occur in liverwort oil bodies? (3) Have the oil bodies of liverworts followed a completely different evolutionary pathway by evolving a single unit membrane surrounding the OB globules instead of a structural protein? (4) What is the composition of the liverwort oil body membrane? (5) What are the mechanisms of metabolic activity in the oil bodies of liverworts? It will be challenging to deal with such an organelle with largely unknown morphology, development, function, and effects of many of its secondary compounds. One way forward may be to conduct proteomic studies in order to characterize the major proteins of the oil bodies and also identify the proteins that are involved in metabolism. Novel tools for visualizing and exploring oil body origin, development and dynamics, and enhancements of imaging techniques such as high resolution electron microscopy, would likely accelerate the deciphering of the biology and evolution of the oil bodies of the liverworts. We believe that the results would provide important information about the mechanisms for the origin of compartmentalization in liverwort oil bodies and would eventually fill the gap in the understanding of lipid droplet evolution in green plants. Furthermore, the elucidation of liverwort oil body metabolism will provide essential knowledge for potential pharmaceutical applications.

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