

Membrane Lipids, Waxes and Oxylipins in the Moss Model Organism *Physcomitrella patens*

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(Received November 14, 2018; Accepted December 24, 2018)

The moss *Physcomitrella patens* receives increased scientific interest since its genome was sequenced a decade ago. As a bryophyte, it represents the first group of plants that evolved in a terrestrial habitat still without a vascular system that developed later in tracheophytes. It is easily transformable via homologous recombination, which enables the formation of targeted loss-of-function mutants. Even though genetics, development and life cycle in *Physcomitrella* are well studied nowadays, research on lipids in *Physcomitrella* is still underdeveloped. This review aims on presenting an overview on the state of the art of lipid research with a focus on membrane lipids, surface lipids and oxylipins. We discuss in this review that *Physcomitrella* possesses very interesting features regarding its membrane lipids. Here, the presence of very-long-chain polyunsaturated fatty acids (VLC-PUFA) still shows a closer similarity to marine microalgae than to vascular plants. Unlike algae, *Physcomitrella* has a cuticle comparable to vascular plants composed of cutin and waxes. The presence of VLC-PUFA in *Physcomitrella* also leads to a greater variability of signaling lipids even though the phytohormone jasmonic acid is not present in this organism, which is different to vascular plants. In summary, the research on lipids in *Physcomitrella* is still in its infancy, especially considering membrane lipids. We hope that this review will help to promote the further advancement of lipid research in this important model organism in the future, so we can better understand how lipids are involved in the evolution of land plants.

Keywords: Bryophytes • Membrane lipids • Oxylipins • *Physcomitrella patens* • Waxes.

Introduction

Bryophytes, which include mosses, liverworts and hornworts, are a division of land plants with origins close to the earliest land-colonizing plants, which evolved about 450 million years ago (Clarke et al. 2011). They represent the evolutionary link between marine plants, like algae, and later terrestrial vascular plants. Plant biology, however, focusses still primarily on vascular plants, especially seed plants. Bryophytes represent a different strategy for dealing with terrestrial conditions (Cuming

2012), which also reflects upon their molecular makeup, including lipids. Identifying the differences in lipid metabolism and function between bryophytes and vascular plants therefore can provide us with insights into these two types of plants developed differently over time on a molecular level. Furthermore, understanding the lipid composition of bryophytes could also give us insights into how marine organisms developed the capability to grow on land.

Of all bryophytes, the moss *Physcomitrella patens* is possibly the best studied species so far (Cove 2005). It was chosen as a model organism and its genome was sequenced in 2008 (Rensing et al. 2008). However, by growing it at room temperature and long-day growth conditions (16 h light/8 h dark), as well as mechanically disrupting the moss tissue regularly, *P. patens* cultures can be maintained indefinitely without the need for spore production. The 'Gransden' strain of *P. patens*, the most commonly used strain in studies, has been grown continuously under laboratory conditions for over half a century now (Ashton and Cove 1977), which apparently resulted in a reduced general fertility compared to moss grown in nature (Schaefer and Zrýd 2001). Genetic modification is, compared to most other plant model organism, much easier when working with *P. patens* (Frank et al. 2005). The organism possesses a highly effective DNA repair mechanism, allowing transformation of protoplasts via homologous recombination with an effectiveness similar to the yeast *Saccharomyces cerevisiae* (Schaefer et al. 1991). This feature, combined with a haploid main growth stage, makes it much easier in *P. patens* to create targeted loss-of-function mutants than compared to vascular model plants like *Arabidopsis thaliana*. Interestingly, one of the first mutants that had been generated in *P. patens* was a loss-of-function mutant in a $\Delta 6$ desaturase affecting membrane lipid metabolism (Girke et al. 1998).

Compared to higher plants the life cycle of the *P. patens* Gransden strain completes in 3–4 months and the moss grows in a rather simple developmental pattern (Engel 1968). The dominant phase of the moss is the haploid gametophyte. The cycle starts either via vegetative propagation from protoplasts and from disrupted tissue or via reproductive growth from spores (Cove et al. 2006). The differentiation into the various developmental stages is phytohormone-dependent (Decker et al. 2006). The germinating spore or the protoplast differentiates into the protonemal tissue (Fig. 1A). The protonema is

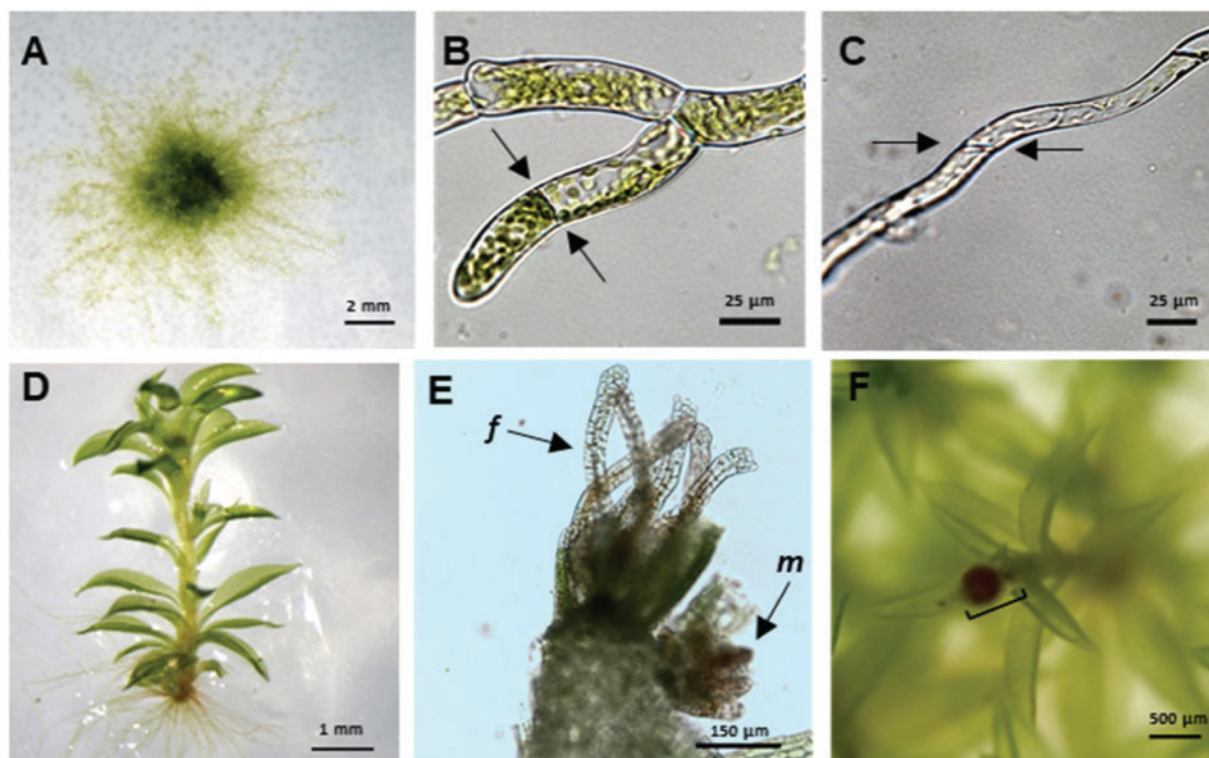


Fig. 1 Different developmental stages of *P. patens*. (A) A 3-week-old colony showing protonema growth that consists of chloronemal cells in the center and caulonemal cells in the periphery. (B) Chloronemal filaments with a characteristic cross wall oriented perpendicular to the cell axis (arrows). (C) Caulonemal filaments with a characteristic cross wall oriented oblique to the cell axis (arrows). (D) A leafy shoot, the gametophore, build-up of a stem, leaf-like organs, the phyllids and root-like organs, the rhizoids. (E) Close-up of the top of a gametophore, carrying the gametangia: the female archegonia (*f*) and the male antheridia (*m*) (arrows). (F) Close-up of the top of a gametophore carrying the sporophyte with a mature spore capsule.

the juvenile stage of the moss and grows in a network of filamentous cells composed of two different cell types: the assimilatory chloronemata (Fig. 1B) and the adventitious caulonemata (Fig. 1C) (Reski 1998). The chloronemal cells are the first cells that develop from the germinating spore. They contain many chloroplasts and are rather short. The chloronemal cells eventually give rise to the caulonemata. In contrast to the chloronemata the caulonemal cells are longer, thinner and contain fewer, less developed chloroplasts. Generation of side branches into secondary chloronemal and caulonemal cells leads to a interwoven filamentous protonemal network. The two described cell types can be identified by the characteristic orientation of the cross walls between two filaments to the cell axis. In chloronemal cells the cross wall orients perpendicular to the cell axis (Fig. 1B) while in caulonemal cells the cross wall shows an oblique orientation to the cell axis (Fig. 1C) (Reski 1998). The growth of the protonemal filaments happens by a serial division of the apical cells. Some of the side branches formed by the subapical cells develop into bud formation that eventually give rise to the adult structure of the moss. The emerging leafy shoot is the gametophore (Fig. 1D). The gametophore has a stem, leaf-like organs called phyllids and root-like organs called rhizoids (Reski 1998). Application of autumn-like conditions, meaning water overlay, short-day conditions (8 h light/16 h dark) and a temperature shift below

18°C, induces the formation of female and male gametangia, archegonia and antheridia, respectively, on top of the gametophore (Fig. 1E) (Engel 1968, Hohe et al. 2002). Thanks to the moist environment, the motile male gametes (spermatozoids) can move from the male antheridia to the female archegonia. After fertilization, the spore capsule develops (Fig. 1F). This stage of the life cycle is the sporophyte, representing the only diploid stage in the moss development. After the spore capsule releases the haploid spores, the cycle starts over again.

Even though *P. patens* has been established as a moss model organism for several years, many areas of research remain understudied compared to other model plants. Lipids in *P. patens* have so far been analyzed only scarcely, with a focus on primary lipid compounds like fatty acids (FA) or some lipid-derived signaling compounds. Lipid research in *P. patens* remains a niche topic, though the organism contains like other bryophytes some interesting differences in lipid composition compared to seed plants. Recently, the liverwort *Marchantia polymorpha* has emerged as new bryophyte model organism (Ishizaki et al. 2016), which could also lead to insights into moss lipid composition in the near future. Though specific research into lipid composition of bryophytes is still limited, it can be assumed that the unique features of these early plants are connected to their lipid composition as well (Dembitsky 1993). Mosses make up substantial parts of the biomass in cold

climate zones, like arctic tundra and boreal woodlands, where vascular plants are far less viable (Rütten and Santarius 1992). Mosses can survive complete desiccation (Proctor et al. 2007) and frozen hibernation for thousands of years (Roads et al. 2014). The adaptation to low temperatures and reduced availability of water has been linked to lipids in vascular plants many times (Hincha and Zuther 2014, Ohlrogge et al. 2015), since these abiotic stresses also affect agriculture a lot (Bohnert and Sheveleva 1998). Studying how bryophytes acquired their high tolerance to abiotic stresses on a level of lipid composition could also lead to valuable insights into how crop plants could be modified to deal with these conditions through genetic engineering.

In this review, we will summarize the current state of the art in lipid research of *P. patens* since the last comprehensive review on bryophyte lipids (Dembitsky 1993). Moreover, we will discuss first insights into the function of lipids in this moss.

Membrane Lipids

Fatty acids

FA represent the basic building block of most lipids. They consist of a long hydrocarbon chain (with variable length) and a

carboxy head group at one end of the chain. Nomenclature of FA is commonly as follows: X:Y, with X representing the number of carbon atoms and Y the number of double bonds inside the carbon chain. The position of double bonds is often specified by either giving an exact position inside the carbon chain ($\Delta\#$, counting from the carboxy head group) or giving the position of the last double bond ($n\#$, counting from the methyl end of the carbon chain) with additional double bonds normally being located in intervals of three carbon atoms closer to the carboxy head group.

All bryophytes, including *P. patens*, are different from seed plants in that they contain high amounts of very-long-chain polyunsaturated FA (VLC-PUFA), harboring 20 or 22 carbon atoms. In *P. patens*, one of the principal PUFA in all membrane lipid classes is arachidonic acid (ARA), 20:4 $\Delta^{5,8,11,14}$. Other major FAs in *P. patens* are comparable to those of seed plants, including 16:3 n -3, 18:2 n -6 and 18:3 n -3 in glycolipids, and 16:0, 18:2 n -6 and 18:3 n -3 in phospholipids (Fig. 2A) (Grimsley et al. 1981). ARA was detected in other moss species as well, alongside the even further desaturated VLC-PUFA 20:5 n -3 (Dembitsky 1993). Knockout mutants of *P. patens* that are unable to synthesize 20:4, specifically of the Δ 6-desaturase (Girke et al. 1998), of the Δ 5-desaturase (Kaewsuwan et al. 2006), and of the Δ 6-elongase (Zank et al. 2002, Eiamsa-

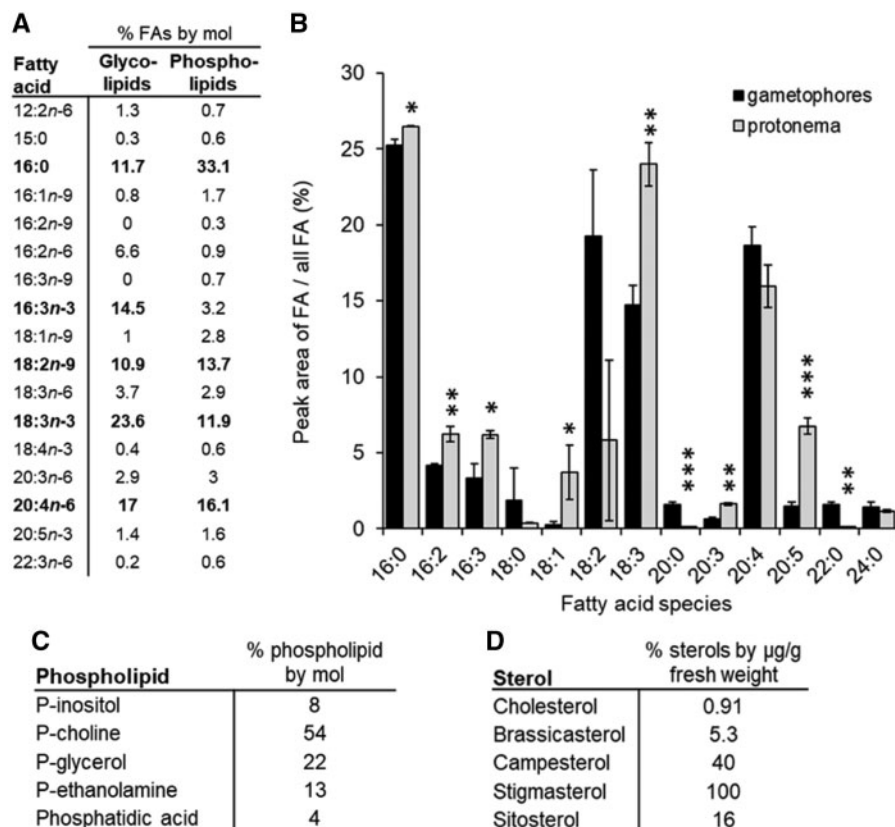


Fig. 2 Composition of various groups of membrane lipids and tissue types in *P. patens*. (A) FA composition in glycolipids and phospholipids of *P. patens* (Grimsley 1981). Bold letters mark major FA with >10% relative abundance. (B) FA profile in different tissue types of *P. patens* (Beike et al. 2014). Asterisks represent results of a student *t*-test (* P < 0.05, ** P < 0.01, *** P < 0.001). (C) Distribution of head groups in phospholipids of *P. patens* (Grimsley 1981). (D) Distribution of steroid core structures in *P. patens* (Morikawa et al. 2009).

Ard et al. 2013), do not exhibit any visible phenotype under normal or stressed growth conditions. VLC-PUFA are commonly also detected in microalgae (Bigogno et al. 2002, Harwood and Guschina 2009), which could mean that VLC-PUFA in bryophytes are a remnant from their marine origin and ultimately not crucial for survival under terrestrial conditions. VLC-PUFA in bryophytes could also fulfill additional functions in nature that are not needed for mosses grown at laboratory conditions, but such theories remain speculative. Other enzymes involved in FA biosynthesis have not been described for *P. patens* yet but the acyl:coenzyme A synthetase (ACS) gene family has been at least fully annotated (de Azevedo Souza et al. 2008).

In *P. patens*, the different growth stages of the gametophore (filamentous protonema and leaf-like gametophyte tissue) do not differ significantly from each other in their FA profile (Fig. 2B) (Beike et al. 2014). There are slight differences in that gametophyte tissue contains less 18:3 and 20:5 FAs compared to protonema tissue, with a subsequent increase of 18:2 (Fig. 2B).

A first hint that the desaturation of FA may be involved in the cold stress response in the moss as well was provided recently by identifying the cold-induced expression of a transcript for a yet unknown desaturase (Beike et al. 2015).

Polar glycerolipids

Glycerolipids are the most common type of membrane lipids in most organisms, consisting of a glycerol backbone to which usually two FA and a polar head group are attached to. The type of polar head group defines the function and name of that specific lipid class. Phospholipids, which possess different types of head groups that all base on phosphate, are found in almost all types of membranes. Glycoglycerolipids are exclusively found in plants and make up the majority of lipids found in plastids (Ohlrogge et al. 2015).

Research on molecular composition of glycerolipids in *P. patens* is scarce, being limited to mostly FA profiles of broad lipid classes, like glycolipids, phospholipids and neutral lipids (Grimsley et al. 1981). Phospholipids were analyzed a degree more thoroughly, separating contents of different phospholipid subclasses (Fig. 2C) (Grimsley et al. 1981). *Physcomitrella patens* phospholipids are mostly composed of phosphatidyl(P)-choline (54%) and P-glycerol (22%), and lesser amounts of P-ethanolamine, P-inositol and phosphatidic acid. This is largely similar to what was found for *A. thaliana*, with the main difference that P-choline and P-glycerol are present in *A. thaliana* in about equal amounts (Welti et al. 2002). However, other publications found that P-ethanolamine is present in higher amounts in *A. thaliana* lipids (Browse et al. 1986).

Other lipid classes were not analyzed in extent, however, it is known that the glycolipid class monogalactosyl-diacylglycerol and betaine lipids are present in other classes of bryophytes (Dembitsky 1993). Betaines are zwitterionic non-phosphorous lipids with similar functions as phospholipids and are known from marine microalgae to play a major role in the composition of lipids (Eichenberger et al. 1993, Cañavate et al. 2016). The

presence of these algae-typical lipids in bryophytes could be attributed to the fact these plants are still partially adapted to living under conditions that marine organisms favor.

Sterols

Sterols represent a lipid class that is not formed from FA but from isopentenyl pyrophosphate instead. As in all eukaryotes, sterols in plants are necessary for survival (Ohlrogge et al. 2015). As part of the plasma membrane, they are important for establishing membrane fluidity in concert with other lipid classes. In *P. patens*, free sterols were analyzed (Morikawa et al. 2009), with stigmasterol being the most prominent (Fig. 2D). Other major sterols are campesterol (40%) and sitosterol (16%), brassicasterol and cholesterol are only present in minor amounts. It is noteworthy that in vascular plants, the sterol species sitosterol is only present in minor amounts (Schaeffer et al. 2001), while it was detected in all analyzed bryophytes so far (Dembitsky 1993). Complex sterols (glycosylated or acylated) were not analyzed in *P. patens* to this date.

Sphingolipids

Sphingolipids represent a complex lipid class with a variety of confirmed and assumed functions in plants (Markham et al. 2013). In general, sphingolipids are only scarcely studied in all bryophytes. The sphingolipid class glycosyl-inositol-phosphoryl ceramide, which is common in vascular plants like *A. thaliana* (Markham and Jaworski 2007), was detected in a comparative study of different plant species (Buré et al. 2014), but no molecular species analysis was performed till now. There has no other study on sphingolipids in *P. patens* been published to date.

Storage Lipids and Lipid Droplets

In vascular plants, neutral lipids serve as storage molecules mostly in seeds. These storage lipids consist mainly of lipids without polar head groups, such as triacylglycerols and sterol esters. The exact composition of sterol esters or triacylglycerols has to this day not been analyzed in *P. patens*, but it is known that bryophytes in general can contain a relatively high amount of triacylglycerols in green tissues, especially compared to vascular plants. In some bryophytes, like *Ceratodon purpureus*, the overall lipid content can be composed of more than 70% triacylglycerols (Dembitsky 1993). However, it is not clear if *P. patens* can have a similar makeup of storage lipids.

Storage lipids are deposited in eukaryotes in so-called oil bodies or lipid droplets (LD). They are surrounded by a monolayer of membrane lipids, which also inhabit several specific types of proteins (Chapman et al. 2012, Huang 2018). In plants, LD can be found in all tissue types, but accumulate in seed and pollen. LD in seeds are also defined by the presence of oleosins, the most abundant type of LD specific proteins, which are commonly only found in this type of tissue (Chapman et al. 2012, Huang 2018). The research on LD of *P. patens*, as for all bryophytes, is limited. It was however discovered that LD from *P. patens* vegetative gametophyte tissue contains oleosins

(Huang et al. 2009), hinting towards some discrepancy between LD of vascular plants and bryophytes. *Physcomitrella patens* was also the evolutionary most ancient plant reported to contain oleosins (Huang et al. 2009), since these types of proteins have so far not been reported in marine plants like microalgae. The composition of lipids in the LD of *P. patens* is mostly similar to that of *A. thaliana* vegetative tissue, containing mostly triacylglycerols and sterol esters, but no molecular analysis of these lipids has been conducted so far (Huang et al. 2009).

Surface Lipids

Bryophytes are concluded to be the first land plants, thus they had to adapt to live in non-aqueous environment. To protect themselves from desiccation, UV radiation and pathogens they developed a hydrophobic protective surface layer—the cuticle. Although some green algae, like *Coleochaete*, are covered with a hydrophobic cuticle-like layer, bryophytes are the first organisms that developed a cuticle similar to that of flowering plants (Cook and Graham 1998, Yeats et al. 2014).

The aerial parts of *P. patens*—gametophores and spores—are covered with a cuticle, composed of a very hydrophobic polymer cutin and cuticular waxes (Buda et al. 2013). In flowering plants, cutin is mostly built of hydroxyl FA and of small amounts of phenyl compounds. They are linked together via ester bonds, forming the scaffold of the cuticle (Franke et al. 2005, Fich et al. 2016). Waxes are a group of components such as alkanes, aldehydes, saturated FA, ketones, fatty alcohols and wax esters which are covering and sealing the cutin and consequently the plant surface (Rashotte et al. 2001, Samuels et al. 2008). Cutin as well as waxes are synthesized in the ER and afterwards transported through the cell wall to cover the plant surface (Kunst and Samuels 2003). Many studies considering cuticle structure and biosynthesis were performed in seed plants, especially in *A. thaliana*, but relatively little is known about this structure in mosses.

Cutin

Cutin of *P. patens* is very rich in phenolic compounds, especially in *m*- and *p*-coumaric and caffeic acid, but does however not contain ferulic acid, the most abundant phenolic compound found in the cutin of *A. thaliana* (Riley and Kolattukudy 1975, Rautengarten et al. 2012, Buda et al. 2013). An enzyme catalyzing the formation of caffeic acid was characterized as a cytochrome P450 belonging to CYP98 family (*PpCYP98*) (Renault et al. 2017). Enzymes from the CYP98 family, in higher plants, are involved in the phenylpropanoid pathway, a source of metabolites required for lignin biosynthesis (Schoch et al. 2006, Fraser and Chapple 2011). Mutants of *PpCYP98* were showing stunted growth and aborted development of gametophores. This can be the result of organ fusion caused by a low cutin content and a lack of caffeic acid (Renault et al. 2017). As mentioned, the CYP98 enzyme family is involved in biosynthesis of major monolignols that are not present in bryophytes. It was therefore proposed that the pre-lignin pathway is crucial for cuticle formation in *P. patens*. This biosynthesis pathway

leading to a high amount of phenolic compounds, which are important for the cuticle structure of *P. patens*, suggests that there is a common ancestor of the formation of cutin, lignin and also suberin, the latter one being a polymer rich in phenolic compounds present only in seed plants (Renault et al. 2017). This ancestral structure could be the first and critical hydrophobic layer necessary for the transition from a water to a land environment. Another example of plants adapting to land conditions is sporopollenin, a polymer that is covering the pollen of flowering plants (Piffanelli et al. 1998, Ariizumi and Toriyama 2011). Despite the fact that sporopollenin was not found in *P. patens*, an enzyme involved in its biosynthesis (the type III polyketide synthase—PpORS) was recently characterized in this organism (Li et al. 2018). It is closely related to FA synthases involved in cutin and wax biosynthesis in seed plants. Mutants of *PpORS* showed malformed phyllids (leaf-like structures) and could not recover after desiccation. It was suggested that mutants of *PpORS* have a compromised or defective cuticle and it may therefore be that the sporopollenin synthesis pathway also evolved from the same ancestor as the cuticle pathway (Li et al. 2018).

Waxes

In *P. patens*, not only the composition of cutin, but also of waxes differs from that of seed plants. Wax extracted from gametophores of *P. patens* contains very high amounts of fatty alcohols and wax esters, whereas in seed plants the most abundant group are alkanes (Buda et al. 2013). In *P. patens* none of the enzymes involved in wax biosynthesis were characterized, but one of the wax transporters, the ATP-binding cassette transporter *PpABCG7*, was described. *PpABCG7* is a homolog of a previously characterized *ABCG12* transporter in *A. thaliana* (Buda et al. 2013). Mutants of *PpABCG7* formed smaller gametophores which contained less wax but the same amount of cutin and their sporophytes exhibited stunted growth. However, complementation of the *A. thaliana* mutant *abcg12* with *PpABCG7* was not successful, suggesting that due to the differences in the wax composition, those two transporters might have different specificities (Buda et al. 2013).

Physcomitrella patens has a cuticle that is in principal similar in architecture and function to that of flowering plants, however its composition differs. Those differences could be a consequence of different properties between the cuticle of *P. patens* and cuticle of flowering plants. The cuticle of *P. patens* is covering only one cell layer and may need to be more permeable for an increased gas exchange in this non-vascular plant. In addition, cutin in *P. patens* is highly enriched in phenolic compounds, which might be a good protection against UV radiation, one of the crucial features in land colonization. There is evidence suggesting the existence of a hydrophobic layer in first land plants being a common ancestor of the formation of cutin, suberin, lignin and also sporopollenin (Renault et al. 2017, Li et al. 2018). This ancient hydrophobic layer might be a crucial trait for land colonization, but its evolution and adaptation to land environment remains unknown.

Signaling Lipids

Oxylipins

Unsaturated FA can be oxidized, either through spontaneous chemical reaction or catalyzed by certain enzymes (Wasternack and Feussner 2018). The term 'oxylipin' describes in plants mostly those oxidized FA that derive from the reaction catalyzed by lipoxygenases (LOX). These enzymes belong to the class of non-heme dioxygenases and convert PUFA into the corresponding PUFA hydroperoxides (Liavonchanka and Feussner 2006). The hydroperoxides may then act as precursors for a variety of different oxylipin compounds (Mosblech et al. 2009). Oxylipins can fulfill a variety of different functions, but they are usually associated with signaling and stress responses in plants (Laudert and Weiler 1998, Park et al. 2002). The phytohormone jasmonic acid (JA), an important regulator in development and defense against herbivores in plants, directly derives from this oxylipin pathway (Vick and Zimmerman 1984, Wasternack and Hause 2013).

In contrast to membrane lipids and wax esters, oxylipins in *P. patens* have been described in comparatively high detail. Nevertheless, compared to vascular plants, research on bryophyte oxylipins is still limited. A survey of lipids in a great variety of mosses (not including *P. patens*) implies a presence of volatile compounds in bryophytes, especially fatty alcohols and aldehydes (Dembitsky 1993). A main difference between oxylipins in bryophytes and vascular plants is, similar to membrane lipids, the presence of VLC-PUFA in bryophytes, which allows consequently also a greater variety of oxylipins.

In *P. patens* itself, research on oxylipins has been in general more extensive and detailed than in other bryophytes. This includes the study of enzymes involved in oxylipin formation. *Physcomitrella patens* contains seven active variants of LOX enzymes, two of which (LOX1-2) have a high affinity towards the VLC-PUFA 20:4n-6, while the others (LOX3-7) prefer 18:3n-3 as a substrate (Senger et al. 2005, Anterola et al. 2009). Interestingly, similar reactions have been described later for *M. polymorpha* (Kanamoto et al. 2012, Kihara et al. 2014). The reactions catalyzed by these enzymes lead to the formation of different oxylipin species (Stumpe et al. 2010) (Fig. 3A, B). Oxidation of 20:4 may result in the formation of two types of cleavage products, either short-chain alcohols or aldehydes of varying carbon chain length (Wichard et al. 2005). Although the formation of the former octenols has been described in moss and fungi, their synthesis follows different pathways in these two kingdoms. While they derive from 20:4 in the moss by the activity of the described two specific LOX forms, fungi synthesize them from unsaturated C-18 FA by the action of so-called Ppo enzymes [precocious sexual inducer (psi)-producing oxygenases] which are fusion proteins that consist of two heme groups and they belong to the family of P450 enzymes (Brodhun et al. 2010). Interestingly, the pathway found in the moss is closely related to a reaction that has been described in porcine leukocytes (Glasgow et al. 1986). In contrast, the formation of the aldehydes follows a route known from flowering plants via the enzyme hydroperoxide lyase (HPL) which again

belongs to the family of P450 enzymes (Stumpe et al. 2006). However, for none of these short-chain oxylipins a function has been found till today, but several LOX proteins as well as the HPL have been found to be induced upon cold stress (Wang et al. 2009).

The phytohormone JA, which is found in all vascular plants, however seems to be missing from non-vascular plants, including *P. patens*. There are reports of JA being present in some bryophytes (Drábková et al. 2015) and in a terrestrial alga (Hori et al. 2014), but these findings need further evaluation and cannot yet be considered as fully established, especially since genes necessary for JA synthesis and perception were either not yet described or reportedly are not present in the organism (Hori et al. 2014). In *P. patens*, the metabolic pathway for synthesizing JA is established up to the point of its plastidic precursor 12-oxo-phytodienoic acid (12-OPDA), and the enzymes allene oxide synthase (AOS) and allene oxide cyclase (AOC), which are necessary for the synthesis, are present in this organism as well (Bandara et al. 2009, Stumpe et al. 2010, Hashimoto et al. 2011, Scholz et al. 2012). In *P. patens* two enzymes exist that catalyze each step. While PpAOS1 is highly active with both C-18 and C-20-hydroperoxy PUFA as substrates, PpAOS2 is fully active only with C-20-substrates (Scholz et al. 2012). Consequently, only the *Ppaos1* mutant shows a significant reduction in 12-OPDA formation. Interestingly, in case of the two AOC forms a similar picture emerges. PpAOC1 is highly active with the 18:3-derived allene oxide as substrate, while PpAOC2 is fully active with both the 18:3- and 20:4-derived allene oxides (Neumann et al. 2012). Similar findings for these enzymes were reported for other non-vascular plants (Koeduka et al. 2015, Yamamoto et al. 2015, Pratiwi et al. 2017), with JA being absent in the liverwort *M. polymorpha* and the lycophyte *Selaginella moellendorffii*. Interestingly, mutations in either of the two AOC genes lead to reduced fertility and altered sporophyte morphology in *P. patens* (Stumpe et al. 2010). This points to an evolutionary conserved function of cyclopentenone-derived oxylipins in non-vascular and vascular plants, since similar mutations in flowering plants lead to sterile reproductive organs (Laudert et al. 1996, von Malek et al. 2002, Wasternack and Hause 2013). Another approach to analyze the function of 12-OPDA was the proteomic analysis of proteins being induced upon exogenous application with this substance (Toshima et al. 2014). This study confirmed an observation made in *Arabidopsis*: the biosynthesis of cyclopentenones is regulated by a feed-forward loop, because 12-OPDA treatment led to an increase in PpAOC1. In addition, first hints are published that 12-OPDA may be involved in regulating defense responses in the moss as well (Oliver et al. 2009). The receptor COI1 which senses the active compound JA-Ile in vascular plants, however, seems to be present in non-vascular plants (Han 2017) and could interact there with other compounds deriving from OPDA. For *Marchantia* it has been recently shown that the C-16 derivative of 12-OPDA, *dinor*-OPDA is its ligand (Monte et al. 2018). Furthermore, the authors speculate that receptor and ligand in plants co-evolved to better fit the more complex

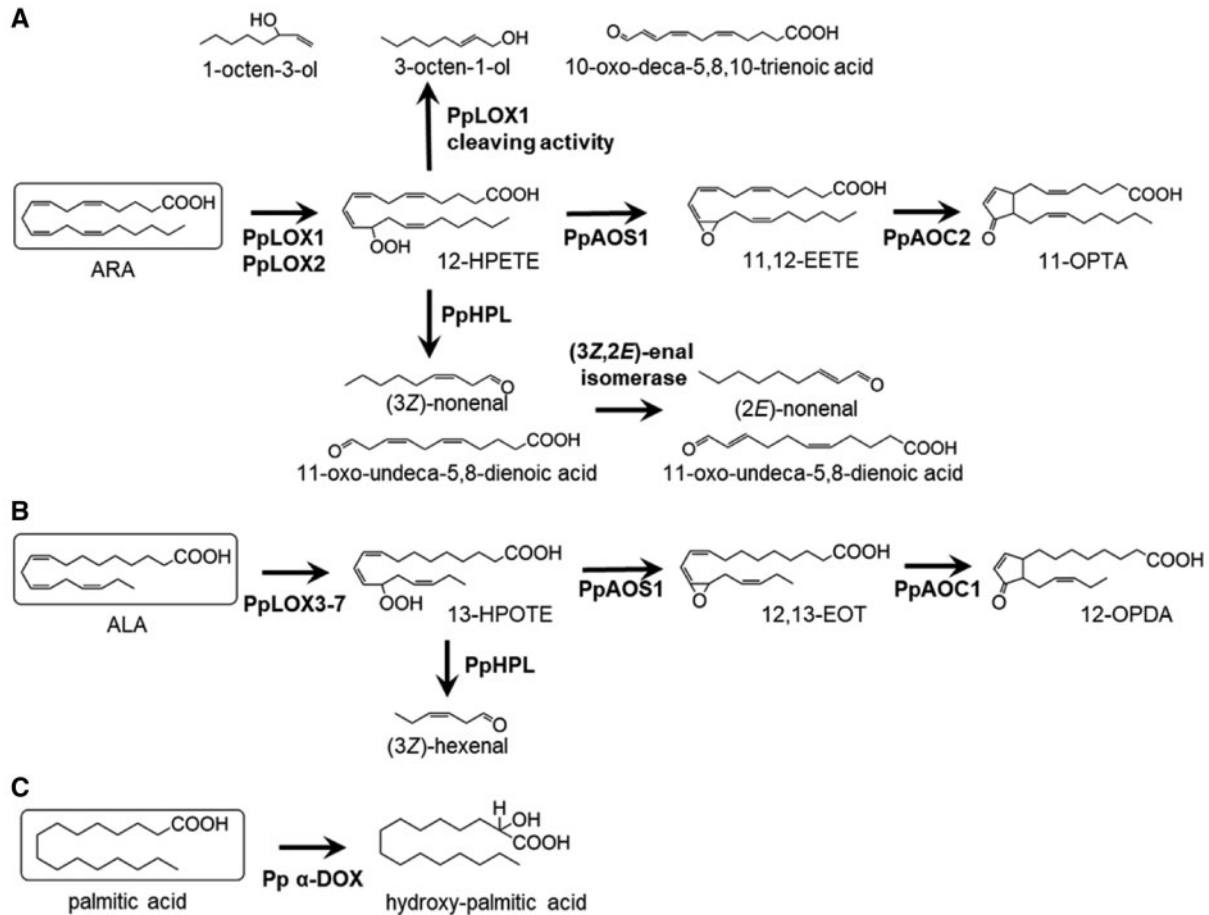


Fig. 3 Oxylipin metabolism in *P. patens*. (A) ARA-dependent LOX pathway. (B) ALA-dependent LOX pathway. (C) Preferred α -DOX reaction in *P. patens*. ARA, arachidonic acid (20:4*n*-6); HPETE, hydroperoxy eicosatetraenoic acid; EETE, epoxy-eicosatetraenoic acid; OPTA, oxo-prosta-trienoic acid; ALA, α -linoleic acid (18:3*n*-3); HPOTE, hydroperoxy octadecatrienoic acid; EOT, epoxy octadecatrienoic acid; 12-OPDA, 12-oxo-phytyldienoic acid; LOX, lipoxygenase; HPL, hydroperoxide lyase; AOS, allene oxide synthase; AOC, allene oxide cyclase; α -DOX, α -dioxygenase.

physiology of vascular plants. It remains unclear, however, in which plant organism JA first appears or if other phytohormones, like 12-OPDA, would fulfill similar functions in early non-vascular plants.

Physcomitrella patens also harbors a variant of the enzyme α -dioxygenase (α -DOX), which in vascular plants can α -oxygenate FAs (Fig. 3C) (Hamberg et al. 2005). Products of this enzyme may be involved in regulating both developmental processes and defense responses (Machado et al. 2015, Ponce De León et al. 2015, Alvarez et al. 2016).

Phosphoinositides

Phosphoinositides have not only a function as membrane lipids being involved in controlling cell polarity, membrane trafficking, ion channels and the cytoskeleton, but serve in addition as signaling molecules (Heilmann and Heilmann 2015). By analyzing the role of two phosphatidylinositol phosphate kinases that phosphorylate the inositol head group leading to the formation of phosphoinositol bisphosphates, it turned out that these highly phosphorylated lipid molecules play a crucial role in the regulation of tip growth in rhizoids (Saavedra et al. 2009, Saavedra et al. 2011). This is similar to what has been shown for

Arabidopsis before (Kusano et al. 2008, Ischebeck et al. 2010). Phosphoinositide-specific phospholipase C cleave these molecules again by generating two second messengers, inositol-1,4,5-trisphosphate (IP3) and diacylglycerol. By analyzing a loss-of-function mutant of PpPLC1, its phenotype revealed a function of IP3 and/or diacylglycerol in regulating the gravity response of the moss (Repp et al. 2004).

Concluding Remarks

Despite the growing interest in *P. patens* on a general scientific level, this moss model organism remains largely unmapped in terms of lipid composition, membrane structure and related fields. Much research on *P. patens* lipids was obtained either several decades ago, or was limited in scale to a few subgroups. However, what is now known about lipid composition of *P. patens* suggests some very interesting differences and similarities in the lipid compositions and functions of mosses and vascular plants on one hand and mosses and microalgae on the other. Hopefully continued research in the future on the molecular composition and tissue specific localization of lipids in *P. patens* will lead to further insights into the evolution of

lipid synthesis and function, membrane structure and signaling from marine plants to vascular plants.

Funding

Deutsche Forschungsgemeinschaft [DFG, IRTG 2172 'PRoTECT' to I.F.]; Göttingen Graduate School of Neurosciences, Biophysics, and Molecular Biology to H.C.R., M.L. and J.G.

Disclosures

The authors have no conflicts of interest to declare.

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