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REVIEW PAPER

Plastid lipid droplets at the crossroads of prenylquinone metabolism

Lucia Eugeni Piller[1], Marion Abraham[1,*], Peter Dörmann[2], Felix Kessler[1,†] and Céline Besagni[1]

[1] Laboratoire de Physiologie Végétale, Université de Neuchâtel, 2000 Neuchâtel, Switzerland
[2] Institute of Molecular Physiology and Biotechnology of Plants (IMBIO), University of Bonn, D-53115 Bonn, Germany

* Present address: Dongseo University, Division of Health Science, Jurye-2Dong, Sasang-gu, 617-764 Busan, South Korea
† To whom correspondence should be addressed. E-mail: felix.kessler@unine.ch

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Abstract

Lipid droplets called plastoglobules (PGs) exist in most plant tissues and plastid types. In chloroplasts, the polar lipid monolayer surrounding these low-density lipoprotein particles is continuous with the outer lipid leaflet of the thylakoid membrane. Often small clusters of two or three PGs, only one of them directly connected to thylakoids, are present. Structural proteins (known as plastid-lipid associated proteins/fibrillins or plastoglobulins) together with lipid metabolic enzymes coat the PGs. The hydrophobic core of PGs contains a range of neutral lipids including the prenylquinones [tocopherols (vitamin E), phylloquinone (vitamin K_1), and plastoquinone (PQ-9)]. In this review the function of PGs and their associated enzymes in prenylquinone metabolism will be discussed.

Key words: Chloroplast prenylquinone metabolism, PG lipid droplets, plastochromanol, plastoquinone, tocopherol, phylloquinone.

Introduction

Plastoglobuli (PGs) were discovered ~40 years ago as osmiophilic globules in electron microscopy of plant tissues (Greenwood *et al.*, 1963; Leggettbailey and Whyborn, 1963). They are present in all tissues and plastid types such as chloroplasts, chromoplasts, and leucoplasts. Easily isolated by flotation density centrifugation, PGs were characterized as low-density globules containing lipids and small amounts of protein (Greenwood *et al.*, 1963; Leggettbailey and Whyborn, 1963; Lichtenthaler and Peveling, 1966; Lichtenthaler, 1968; Kessler and Vidi, 2007).

The first PG protein to be discovered was named fibrillin because it was identified in the carotenoid fibrils of red pepper chromoplasts. Technically, the fibrils are elongated lipid droplets (Deruere *et al.*, 1994). Later, fibrillins were also discovered in association with PGs in leaf tissue and termed plastid-lipid associated proteins (PAPs) or plastoglobulins (Deruere *et al.*, 1994; Pozueta-Romero *et al.*, 1997; Kessler *et al.*, 1999).

Until recently, PGs were largely viewed as passive lipid storage droplets, their size and composition varying as a function of the developmental stage or the type of plastids. However, recent proteome studies of the PGs isolated from *Arabidopsis* chloroplasts and red pepper chromoplasts (Vidi *et al.*, 2006; Ytterberg *et al.*, 2006) revealed the presence not only of an entire family of plastoglobulin proteins but also of enzymes. Many of these are predicted or known to participate in lipid metabolic pathways.

Electron tomographic experiments demonstrated that the PG constitutes a distinct structural and functional subcompartment of the thylakoids. This is underscored by the fact that its hydrophobic core is surrounded by a polar lipid monolayer contiguous with the thylakoid outer lipid leaflet (Austin *et al.*, 2006). PG dimensions range from 30 nm to 5 μm (Lichtenthaler, 1968; Thomson and Platt, 1973; Austin *et al.*, 2006). Several studies demonstrated that under biotic and abiotic stress conditions, the size and number of the lipid droplets increase. Moreover, the PGs may connect, resulting in grape-like clusters (Austin *et al.*, 2006). It has been suggested that PG clusters form by a two-step mechanism:

a primary 'blistering' event at the outer thylakoid lipid leaflet followed by a secondary blistering event at the surface of an existing PG. The resulting connections between PGs and thylakoids as well as those between PGs themselves provide the basis for a bidirectional metabolite conduit between PGs and the thylakoid membrane (Fig. 1).

PGs in various plant species differ with regard to size and number (Lichtenthaler, 2007). In older leaves of herbaceous plants such as spinach, the number of PGs with a small diameter (0.1–0.2 μm) increases to several hundred per chloroplast (Lichtenthaler, 1969). In older, sun-exposed leaves of beech and oak, PGs are less numerous but significantly enlarged (Lichtenthaler, 1968). Moreover, in several-year-old *Ficus* leaves PGs may reach diameters of 0.3–3.0 μm (Lichtenthaler and Weinert, 1970).

Studies of the PG core identified members of the neutral lipid class including prenylquinones, triacylglycerols (TAGs), carotenoid, and others. The prenylquinones, plastoquinol-9 (PQH_2-9) and tocopherol (vitamin E), are among the major constituents of PGs (Lichtenthaler and Peveling, 1966; Tevini and Steinmuller, 1985; Austin *et al.*, 2006; Vidi *et al.*, 2006) whereas phylloquinone (vitamin K_1) is present in minor amounts (Lohmann *et al.*, 2006) (Fig. 1). However, no full lipidome of PGs has been determined so far.

While traces of protein in PGs were observed long ago (Leggettbailey and Whyborn, 1963), it is now known that lipid droplets are coated with specific proteins. Two independent studies reported on the PG proteome, which consists of a total of about three dozen proteins. These belong to three categories: PAPs/fibrillins, chloroplast metabolic enzymes, and unclassified proteins (Vidi *et al.*, 2006; Ytterberg *et al.*, 2006).

The first group contains a total of eight of the 13 member *Arabidopsis* plastoglobulin/PAP/fibrillin family (Vidi *et al.*, 2006; Ytterberg *et al.*, 2006). Fractionation experiments demonstrated the enrichment and physical association of family members, PGL34 (At3g58010) and PGL35 (At4g04020), with PGs (Vidi *et al.*, 2006, 2007). Based on their role in organizing red pepper carotenoid fibrils, the PAPs/fibrillins are hypothesized to fulfil a structural role in PGs too (Deruere *et al.*, 1994). It is interesting to note that the cyanobacterial *Synechocystis* sp. genome also contains two PAP/fibrillin homologues. Mutant analysis demonstrated that they serve, by an unknown process, to protect the organism from photooxidative damage (Cunningham *et al.*, 2010).

The expression of plastoglobulins is regulated in response to abiotic and biotic stress as well as hormone treatment (Brehelin and Kessler, 2008). Cold treatment induced the expression of a plastoglobulin in rice leaves (Lee *et al.*, 2007). AtPGL30.4 (At3g23400) was identified as a phosphorylated protein in the defence response to *Pseudomonas syringae* pv. tomato DC3000 (Jones *et al.*, 2006). In a comparative proteome study, four members of the *Arabidopsis* plastoglobulin family were found to accumulate under high light stress (Giacomelli *et al.*, 2006). Abscisic acid also induced the expression of several plastoglobulins, AtPGL35 in particular (Gillet *et al.*, 1998; Yang *et al.*,

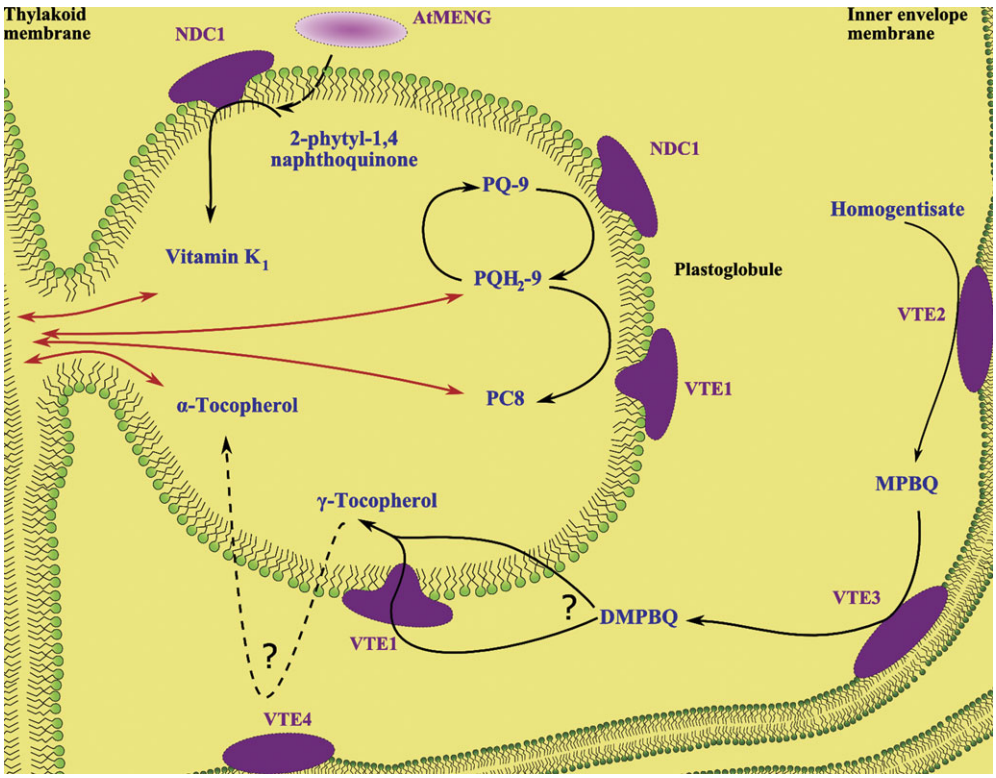

Fig. 1. Function of PG-localized enzymes in prenylquinone metabolism. Prenylquinones are shown in blue, enzymes in violet. Bidirectional trafficking between the PG and the thylakoid membrane is represented by red arrows. PQ-9, plastoquinone; PQH_2-9, plastoquinol; PC8: plastochromanol-8; MPBQ, 2-methyl-6-phytyl-1,4-benzoquinol; DMPBQ, 2,3-dimethyl-5-phytyl-1,4-benzoquinol.

2006). Recently, it has been shown that plastoglobulins accumulate in response to light/cold stress-related jasmonate biosynthesis (Youssef *et al.*, 2010).

The second group of proteins identified in the *Arabidopsis* PG proteome consists of known metabolic enzymes. This category includes the tocopherol cyclase VTE1 (vitamin E defective, At4g32770) involved in vitamin E synthesis, the carotenoid cleavage dioxygenase CCD4 (At4g19170) probably implicated in carotenoid metabolism, the three isoforms of fructose bisphosphate aldolase of the Calvin cycle (At2g21330, At4g38970, and At2g01140), and the allene oxide synthase (AOS) in jasmonate biosynthesis (At5g42650) (Kazan and Manners, 2011).

The third group of PG proteins consists of unclassified proteins. Some of them are predicted to be involved in lipid metabolism. For instance, ELT1 and 2 (esterase/lipase/thioesterase, At1g5440 and At3g26840) may be involved in thylakoid lipid metabolism, while ABC1 (activity of bc1 complex)-like kinases (At1g79600, At4g31390, At5g05200, and At5g71810) are predicted regulators of prenylquinone metabolism (Ytterberg *et al.*, 2006). Thus, the available data strongly suggest that PGs, by the presence of enzymes, intervene in diverse aspects of thylakoid lipid metabolism.

Response of PGs to stress

Under oxidative stress-inducing conditions such as drought, high saline concentration, nitrogen deprivation, high light, viral infection, chilling, and ozone (Nordby and Yelenosky, 1985; Locy *et al.*, 1996; Rey *et al.*, 2000; Oksanen *et al.*, 2001; Gaude *et al.*, 2007; Lichtenthaler, 2007) as well as at different developmental stages (senescence and fruit development) (Kaup *et al.*, 2002), PGs increase in size and number. This occurs in parallel to the disassembly of thylakoid membranes. Also, the lipid composition of PGs will change dramatically due to the accumulation of fatty acid phytyl esters (FAPEs) from thylakoid catabolism (Gaude *et al.*, 2007; Brehelin and Kessler, 2008).

In the thylakoid membrane, reactive oxygen species (ROS) accumulate when the absorption of light by chlorophyll exceeds the capacity for energy utilization by the photosynthetic apparatus (Pospisil, 2011). Photosystem I (PSI) and PSII are the major sites of free radical O_2^- generation. Plant responses against oxidative stress implicate different biochemical pathways including the enhanced synthesis of prenylquinones (Gruszka *et al.*, 2008). Antioxidant action has been attributed to phylloquinone and plastoquinol, although they are primarily known as electron carriers at PSI and PSII, respectively. Tocopherol does not play a role as an electron carrier but is a key antioxidant lipid during high light stress (Munne-Bosch, 2005).

While prenylquinones partly accumulate in PGs, their true site of action is probably the thylakoid membrane where they scavenge ROS and protect the photosystems. Of the proteins in the PG proteome, the tocopherol cyclase VTE1 (Vidi *et al.*, 2006) and the NADPH quinone dehydrogenase C1, NDC1 (Eugeni Piller *et al.*, 2011), are known players in prenylquinone metabolism and implicate PGs as a metabolic compartment.

Implication of PGs in storage and biosynthesis of tocopherols

Tocopherols belong to the amphipathic group of tocochromanols (vitamin E) that also includes tocotrienols (Falk and Munne-Bosch, 2010). The two types of tocochromanols differ in the degree of saturation of their prenyl side chains. Synthesized only in photosynthetic organisms (plants, green algae, and cyanobacteria), tocopherols are composed of a polar region derived from tyrosine and a hydrophobic polyprenyl side chain from the isoprenoid pathway (Fig. 2) (Valentin and Qi, 2005).

The group of tocopherols consists of four different forms, α-, β-, γ-, and δ-, which differ by the number and position of methyl groups on the chromanol ring (Mene-Saffrane and DellaPenna, 2010).

The presence of tocopherols is universal in higher plants, albeit with differential tissue distribution of the various forms: α-tocopherol is predominant in leaves whereas in other organs, such as seeds, flowers, and roots, γ-tocopherol is the principle form (Horvath *et al.*, 2006). In plastids, tocopherol inserts in both the envelope and thylakoid membranes whereby the polar chromanol group faces the hydrophilic surface (Dormann, 2007). Around one-third of the total plastid tocopherol is contained in the *Arabidopsis* PG core (Vidi *et al.*, 2006) (Fig. 1). Under oxidative stress-inducing conditions, such as high light, the production of tocopherols increases to protect membrane lipids from photooxidation and PSII from photoinactivation (DeLong and Steffen, 1997; Havaux *et al.*, 2005). In older leaves under high light stress, the level of α-tocopherol increases >4-fold (Szymanska and Kruk, 2010). The elimination of tocopherols drastically reduces the tolerance of photosynthetic organisms to high light stress (Maeda *et al.*, 2005). The α-tocopherol accumulation correlates with an increase in size and number of PGs in older leaves (Vidi *et al.*, 2006; Brehelin *et al.*, 2007).

Three of the reactions of the tocopherol biosynthesis pathway, mediated by VTE2, VTE3 (Cheng *et al.*, 2003), and VTE4, have been located at the chloroplast inner envelope (Soll *et al.*, 1985). However, surprisingly, the tocopherol cyclase VTE1 (Porfirova *et al.*, 2002) was identified in the PG proteome (Vidi *et al.*, 2006; Ytterberg *et al.*, 2006). Its localization in PGs was confirmed by physical fractionation, immunoelectron microscopy, and expression of a fluorescent fusion protein (Vidi *et al.*, 2006). A serial immunoelectron tomography study revealed the penetration of VTE1 accros of the lipid PG monolayer. This may enable VTE1 to access substrates inside the PGs and carry out the cyclase reaction (Austin *et al.*, 2006).

VTE1 catalyses the conversion of 2,3-dimethyl-5-phytyl-1,4-benzoquinol (DMPBQ) to γ-tocopherol and is required for the formation of the chromanol ring of all tocopherols

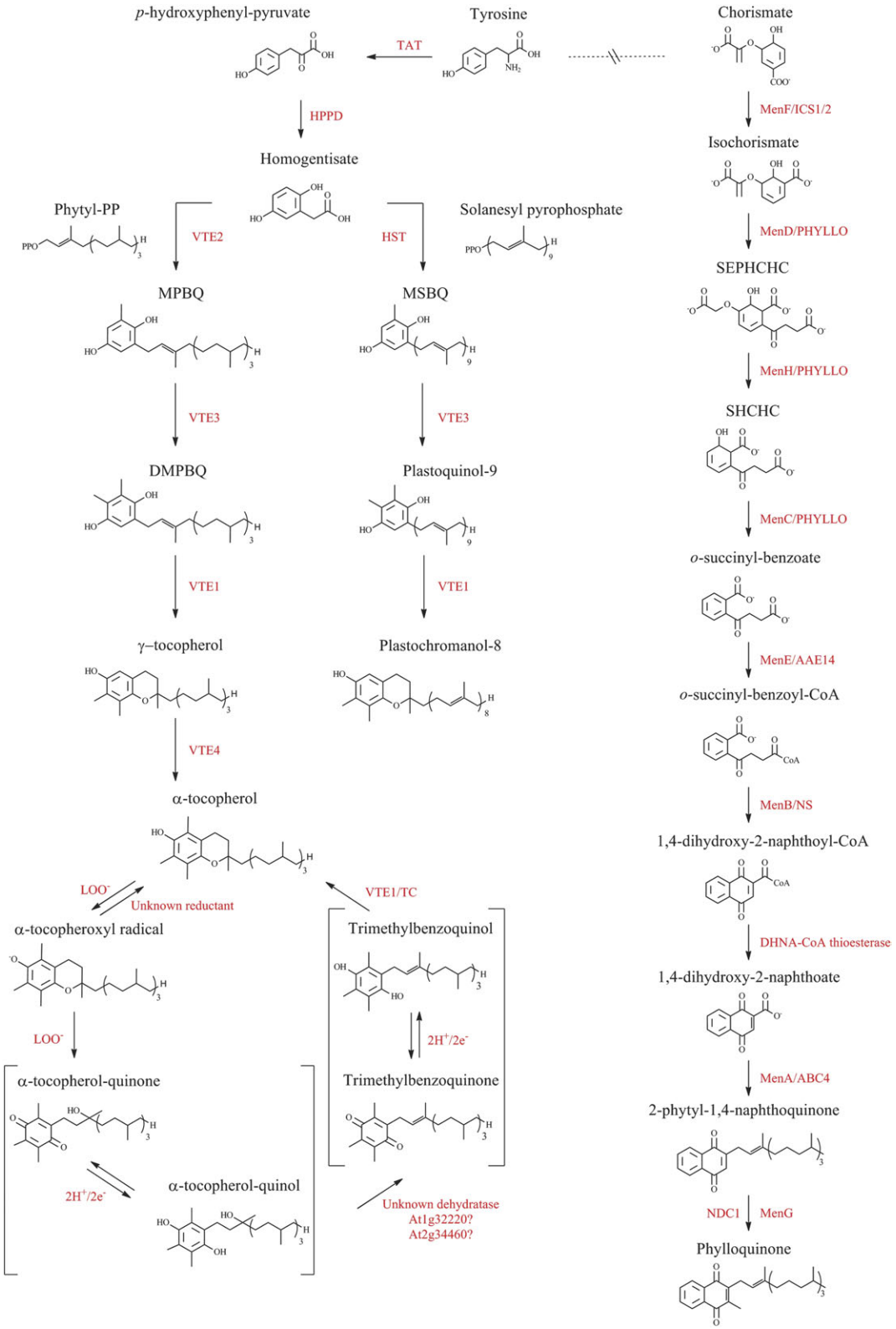

Fig. 2. The biosynthetic pathways of prenylquinones in *Arabidopsis*. Summary of tocopherol, plastoquinol, plastochromanol, and phylloquinone pathways in *Arabidopsis*. The enzyme abbreviations are shown in red. TAT, tyrosine aminotransferase; HPPD, *p*-hydroxyphenyl-pyruvate dioxygenase; HST, homogentisic acid solanesyl transferase; VTE, enzymes of vitamin E synthesis; LOO^{-}, lipid peroxy radical; TC, tocopherol cyclase; Men, menaquinone synthesis; ICS 1/2, isochorismate synthase 1 and 2; AAE14, acyl-CoA activating enzyme isoform 14; NS, naphthoate synthase; DHNA-CoA thioesterase, 1,4-dihydroxy-2-naphthoyl-CoA thioesterase; ECHId, enoyl-CoA hydratase/isomerase; PP, pyrophosphate; MPBQ, 2-methyl-6-phytyl-1,4-benzoquinone; DMPBQ, 2,3-dimethyl-6-phytyl-1,4-benzoquinone; MSBQ, 2-methyl-6-solanesyl-1,4-benzoquinol; PQH_2, plastoquinol; SEPHCHC, 2-succinyl-5-enolpyruvyl-6-hydroxy-3-cyclohexene-1-carboxylate; SHCHC, 2-succinyl-6-hydroxy-2,4-cyclohexadiene-1-carboxylate.

(Soll *et al.*, 1985) (Fig. 2). If VTE1 were uniquely present in PGs, DMPBQ would have to be moved from the inner envelope membrane to PGs where the cyclase would convert it to γ-tocopherol. In support of this hypothesis, DMPBQ was indeed highly enriched in PGs of the *vte1* mutant (Fig. 3).

The last step of α-tocopherol synthesis is carried out by the γ-tocopherol methyl transferase, VTE4, located at the chloroplast envelope (Zbierzak *et al.*, 2010) (Fig. 1). Again, if VTE1 were exclusively located at PGs, γ-tocopherol would have to be transported back to the inner envelope membrane to complete the synthesis of α-tocopherol (Zbierzak *et al.*, 2010). Alternatively, it has been proposed that sufficient VTE1 for vitamin E synthesis may still be present at the envelope membranes and that at PGs VTE1 serves other metabolic purposes such as the recycling of tocopherol oxidation products (DellaPenna and Kobayashi, 2008).

Role of PGs in the tocopherol redox cycle

Tocopherol oxidation products form in response to high light stress (DellaPenna and Kobayashi, 2008). By *in vitro* chemical treatment, 23 different oxidation products can be generated from α- and γ-tocopherol, but only two of these were detected *in vivo*: wild-type plants accumulated α-tocopherol-quinol (α-TQH_2) under high light conditions (DellaPenna and Kobayashi, 2008) and *vte4* mutant plants

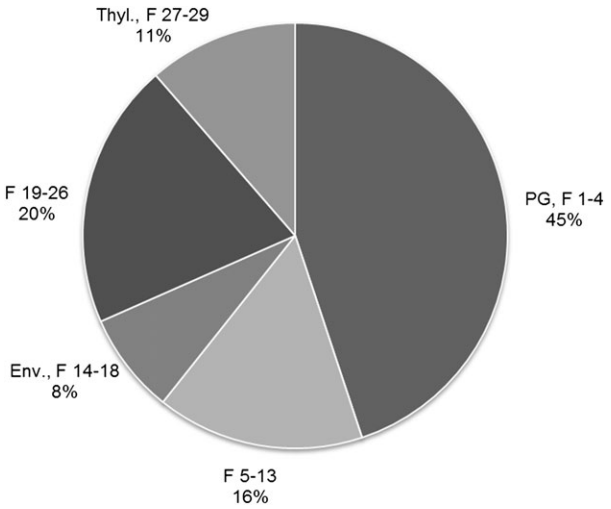

Fig. 3. Enrichment of DMPBQ in *vte1* PGs. DMPBQ (2,3-dimethyl-5-phytyl-1,4-benzoquinol), the precursor of γ-tocopherol, accumulates in the *vte1* mutants. Subplastidial chloroplast fractions were isolated from leaves of 8-week-old *vte1* mutant plants (Vidi *et al.*, 2006). The distribution of DMPBQ (as a percentage of the total) was measured in pooled chloroplast membrane fractions. Fractions F1–4 and F5–13 contained mostly PGs; F14–18 contained envelopes (Env.); F19–26 contained some envelopes and thylakoids; and F27–29 contained thylakoids (Thyl.). The highest amount of DMPBQ is present in the fractions enriched in PGs (~45%).

(containing only γ-tocopherol) accumulated γ-TQH_2. To determine whether α-TQH_2 was degraded or recycled to α-tocopherol, isolated wild-type and *vte1* chloroplasts were incubated with ^{14}C-labelled α-TQH_2 (DellaPenna and Kobayashi, 2008; Mene-Saffrane and DellaPenna, 2010). In wild-type chloroplasts, the incubation led to α-tocopherol accumulation, whereas in *vte1* mutants a substrate of tocopherol cyclase, trimethylphytylbenzoquinone (TMPBQ), was detected (DellaPenna and Kobayashi, 2008). This is clear evidence that tocopherol oxidation products are recycled in higher plants. The α-tocopherol quinone (α-TQ) oxidation product was present in thylakoids, envelope membranes, as well as PGs in chloroplast fractionation experiments (Kruk and Nowicka, 2010).

The proposed α-tocopherol redox cycle starts by a two-step oxidation, with each step characterized by the loss of a single electron (Mene-Saffrane and DellaPenna, 2010) (Fig. 2). In the first step, α-tocopherol is oxidized to the α-tocopherol radical by a lipid peroxy radical ($LOO^{\cdot}$). This product may be reduced back to α-tocopherol by an unknown reductant, possibly ascorbate, or be oxidized further by a second lipid peroxy radical, resulting in the formation of α-TQH_2. To regenerate α-tocopherol, α-TQH_2 must undergo a dehydration step catalysed by an as yet unidentified dehydratase. This will result in TMPBQ, which in turn will undergo the cyclase reaction catalysed by VTE1 and results in the completion of the cycle. With regard to the potential role of PGs in the cycle, not only are they enriched in tocopherol cyclase VTE1 (Austin *et al.*, 2006; Vidi *et al.*, 2006; Ytterberg *et al.*, 2006) but the PG proteome also contains two predicted dehydratases (At2g34460 and At1g32220). In summary, the currently available evidence suggests that PGs participate in the tocopherol recycling pathway.

Implication of VTE1 and NDC1 in plastoquinol metabolism

PQH_2-9 is well known as an electron and proton carrier in the photosynthetic transport chain between PSII and the cytochrome b_6f complex (Muh *et al.*, 2011). However, plastoquinol has also been shown to have a physiological antioxidant activity (Szymanska and Kruk, 2010). Structurally related to α-tocopherol, it exerts a photoprotective role on PSII during high light stress. It does so by scavenging singlet oxygen generated by chlorophyll at the reaction centre (Kruk and Trebst, 2008), thereby inhibiting lipid peroxydation (Hundal *et al.*, 1995). The plastoquinol head group as well as the isoprenoid chain are involved in the process which may confer additional antioxidant power over tocopherols (Gruszka *et al.*, 2008).

Plastoquinone-9 (PQ-9) is associated with Q_A and Q_B sites of PSII but also exists in a free form in thylakoid membranes. Together, these are considered the thylakoid or photoactive PQ pool (Strzalka and Kruk, 1999). However, PQ-9 is also present in a separate pool contained in PGs (Szymanska and Kruk, 2010; Zbierzak *et al.*, 2010) (Fig. 1).

The PG PQ pool is not normally photoactive and does not directly participate in photosynthetic electron flow (Eugeni Piller *et al.*, 2011). It might therefore serve both as a reservoir of antioxidant and to replenish the thylakoid pool (Zbierzak *et al.*, 2010).

In *Arabidopsis* leaves, the level of plastoquinol dramatically increases under high light stress. The reduced form of PQ (PQH_2-9) increased 16- and 9- fold in old and young rosette leaves while the total amount of PQ (reduced plus oxidized) increased 8- and 11- fold, respectively (Szymanska and Kruk, 2010). The majority of the plastoquinol under high light conditions is photosynthetically inactive and accumulates in PGs. It is tempting to speculate that the plastoquinol that is irreversibly degraded by ROS in thylakoid membranes is replaced by plastoquinol from PGs (Szymanska and Kruk, 2010). This may partially explain why PQ accumulates to very high levels under high light conditions. NDC1, the NADPH-dependent quinone dehydrogenase C1, in PG functions to reduce the oxidized proportion of the non-photochemical pool of plastoquinol in PGs. Indeed, the *ndc1* mutant had a significantly higher percentage of oxidized PQ than the wild type (Eugeni Piller *et al.*, 2011). Therefore, NDC1 constitutes a unique electron transport pathway separate from cyclic electron flow mediated by the NAD(P)H dehydrogenase (NDH) complex or the PROTON GRADIENT REGULATION 5 (PGR) pathway (Shikanai, 2007; Peng *et al.*, 2010). The NDC1-mediated electron pathway, however, is probably limited by the availability of PQ inside PGs that cannot rapidly be reoxidized as happens in NDH- and PGR5-dependent cyclic electron flow (Eugeni Piller *et al.*, 2011).

Plastochromanol (PC8), derived from PQH_2-9 by tocopherol cyclase activity, is present in leaves, seeds, and other organs of *Arabidopsis* plants (Mene-Saffrane and Della-Penna, 2010; Szymanska and Kruk, 2010; Zbierzak *et al.*, 2010). It constitutes 5–10% of the total tocochromanol, although this value may be higher in senescing leaves (Szymanska and Kruk, 2010). Around 50% of the PC8 is present in PGs (Zbierzak *et al.*, 2010). Together with γ-tocopherol, PC8 has been shown to be required for efficient germination after longer periods of seed quiescence (Mene-Saffrane and DellaPenna, 2010). The levels of PC8 increase under high light stress as well as in ageing leaves. Several studies have demonstrated that PC8 is an efficient singlet oxygen scavenger (Gruszka *et al.*, 2008) and an inhibitor of lipid peroxidation (Olejnik *et al.*, 1997). Its antioxidant activity is comparable with that of tocopherols (Olejnik *et al.*, 1997). This is not surprising because plastochromanol has a chromanol group identical to that of γ-tocopherol, differing only in the C40 polyunsaturated solanesyl side chain instead of the phytol. Most probably, its source is the PQH_2-9 in the PG pool (Kumar *et al.*, 2005; Kruk and Trebst, 2008) (Fig. 2) where the tocopherol cyclase is also present (Fig. 1).

In the *ndc1* mutant, PC8 was decreased (Eugeni Piller *et al.*, 2011). Most probably, this is linked to the decrease of its direct precursor, PQH_2-9, the substrate of VTE1 (Grutter *et al.*, 2006). In the *vte1* mutant, PC8 formation was entirely abolished but the overexpression of VTE1 induced a 2.4-fold increase of PC8 levels. This resulted in the proliferation of PG numbers and increased cluster formation (Kanwischer *et al.*, 2005; Zbierzak *et al.*, 2010).

Implication of PGs in storage and biosynthesis of phylloquinone via NDC1

Phylloquinone (2-methyl-3-phytyl-1,4-naphthoquinone) or vitamin K_1 is an prenylquinone composed of a naphthoquinone ring and a prenyl side chain derived from phytyl-diphosphate (Fig. 2). It is synthesized in all organisms performing oxygenic photosynthesis. In higher plants, phylloquinone occurs in leaves where it serves as an electron carrier in the quinone/semiquinone turnover in PSI (Joyard *et al.*, 2009). The overall stoichiometry of phylloquinone has been estimated at 3 mol of vitamin K_1 per 1 mol of PSI. However, only two molecules of phylloquinone are present for each PSI complex. This suggests that a separate pool exists that is not associated with PSI (Lohmann *et al.*, 2006; Brehelin and Kessler, 2008). Interestingly, this is in good agreement with the ~30% of the total phylloquinone located in *Arabidopsis* PGs (Lohmann *et al.*, 2006) (Fig. 1).

The enzymatic reactions of phylloquinone biosynthesis take place at the inner membrane of the chloroplast envelope (Schultz *et al.*, 1981), but recent evidence in *Arabidopsis* suggests that peroxisomes may also be implicated. In cyanobacteria and red algae, the pathway is catalysed by Men proteins including successively: MenF, MenD, MenH, MenC, MenE, MenB, MenA, and MenG enzymes. The first step of phylloquinone biosynthesis (MenF) implicates the conversion of chorismate to isochorismate. In *Arabidopsis*, this reaction may be catalysed by two genes, *ICS1* and *ICS2*, showing homology with the *MenF* gene (Ausubel *et al.*, 2001; Gross *et al.*, 2006; Garcion *et al.*, 2008; Metraux *et al.*, 2008).

The double homozygous *ics1 ics2* mutant was completely devoid of phylloquinone, and plants remained smaller and had a pale green or yellowish phenotype compared with the wild type or the single mutants. The two enzymes may form a complex in the chloroplast stroma to facilitate the efficient channelling of intermediates through the pathway (Ausubel *et al.*, 2001; Metraux *et al.*, 2008).

The conversion of isochorismate into *o*-succinyl-benzoate (OSB) implicates three distinct enzymes (Men D, Men H, and Men C) in cyanobacteria. These functions are encoded by the composite gene PHYLLO in *Arabidopsis* (Gross *et al.*, 2006). In cyanobacteria, the conversion of OSB to *o*-succinyl-benzoyl-CoA is catalysed by the ligase MenE. The presence of several MenE homologues in the *Arabidopsis* genome makes it difficult to assign the ligase function: it seems likely, however, that the OSB-CoA ligase corresponds to the acyl-activating enzyme 14 (AAE14) (Browse *et al.*, 2008). The *aae14* mutant is unable to grow on soil due to the lack of phylloquinone. Recently, it has been demonstrated that MenE/AAE14 is dually targeted to both chloroplasts and peroxisomes (Reumann *et al.*, 2010). In the following

step, OSB-CoA is converted to 1,4-dihydroxy-2-naphthoyl-CoA by an enzyme orthologous to MenB, the naphthoate synthase (NS/ECHId) encoded by a single *Arabidopsis* gene (Gross *et al.*, 2006; Browse *et al.*, 2008; Babujee *et al.*, 2010). Prior to these studies, neither functional data nor subcellular localization had been reported for a MenB homologue in plants. Recently, NS/ECHId was localized to the *Arabidopsis* peroxisome (Babujee *et al.*, 2010; Reumann *et al.*, 2010). It has also been proposed that the conversion of 1,4-dihydroxy-2-naphthoyl-CoA to 1,4-dihydroxy-2-naphthoate (DHNA) is catalysed by the 1,4-dihydroxy-2-naphthoyl-CoA (DHNA-CoA) thioesterase and that this enzyme may also be a peroxisomal protein (Reumann, 2004; Reumann *et al.*, 2010). These recent findings suggest that phylloquinone biosynthesis of higher plants is partially compartmentalized in peroxisomes.

The last steps of phylloquinone synthesis involve the attachment of the phytyl chain to DHNA by MenA/ABC4 (Shimada *et al.*, 2005) and the methylation of 2-phytyl-1,4-naphthoquinone by MenG (Lohmann *et al.*, 2006). Genetic analysis of phylloquinone pathway in plants allowed the isolation of the *AtmenA* mutant of *Arabidopsis*, which lacks the DHNA phytyltransferase (Shimada *et al.*, 2005). Total absence of vitamin K_1 in *AtmenA* mutant plants results in a drastic reduction in both growth and the accumulation of PSII and PQ-9.

In contrast, growth and photosynthesis were only slightly affected in *AtmenG* mutant plants raised under normal light conditions. This fact suggests that 2-phytyl-1,4-naphthoquinone can functionally replace phylloquinone as an electron carrier in PSI. Under high light stress, biochemical and physiological studies of the *AtmenG* mutant demonstrated a significant decrease in the level of PSI complexes caused by oxidative damage at the PSI reaction centre. This led to lowered PSII efficiency and negatively affected the performance of the entire photosynthetic electron transfer chain (Lohmann *et al.*, 2006).

Interestingly, a much higher proportion of the 2-phytyl-1,4-naphthoquinone in the *AtmenG* mutant than of phylloquinone in the wild type was present in PGs. A non-targeted lipidomic analysis of *ndc1* mutant plants led to the unexpected discovery that, similar to AtmenG, phylloquinone was almost completely absent and that 2-phytyl-1,4-naphthoquinone accumulated instead (Eugeni Piller *et al.*, 2011). Also, At*MenG* was normally expressed in *ndc1* plants. While this result is difficult to explain, it suggests the implication of NDC1 and PGs in the AtMenG methylation step.

Interestingly, the multifunctional protein PHYLLO and AtMenG gave punctate fluorescence rather than the ring-like fluorescence typical of chloroplast envelope proteins when transiently expressed as green fluorescent protein (GFP) or yellow fluorescent protein (YFP) fusion proteins in *Arabidopsis* protoplasts. As the punctate fluorescence resembles that of NDC1 it may hint at a PG localization of PHYLLO and AtMenG (Gross *et al.*, 2006; Lohmann *et al.*, 2006; Eugeni Piller *et al.*, 2011). However, PHYLLO and AtMenG were not found in the PG proteome. Possibly they are only loosely or transiently associated with PGs and lost during the purification procedure.

Together with the presence of phylloquinone and 2-phytyl-1,4-naphthoquinone, the new role for NDC1 suggests a role for PGs in phylloquinone metabolism.

Conclusions

In the last few years several studies demonstrated the important role of PGs in chloroplast lipid metabolism. The determination of the *Arabidopsis* PG proteome (Vidi *et al.*, 2006; Ytterberg *et al.*, 2006) paved the way to the discovery that PGs do not only store lipids but also actively participate in their synthesis.

The *Arabidopsis* PG proteome contains 34 proteins, assignable to three groups: plastoglobulins, metabolic enzymes, and proteins of unknown function. While many of the unknown proteins are predicted to be enzymes, their function still remains to be discovered.

To discover the function of such candidate enzymes in lipid metabolism, a powerful non-targeted lipidomics analysis can be employed to correlate changes in metabolite profiles with enzyme function.

The final biosynthetic steps of tocopherol require VTE1 and VTE4. VTE1 was localized in PGs while VTE4 was located at the inner envelope membrane. This hints at the interesting possibility of prenylquinone metabolite trafficking inside the chloroplast.

It is still not known whether PGs are structurally required for proper chloroplast function, but forward genetic screens have the potential to provide answers to this question. Proteome analyses of PGs isolated from plants under various stress or developmental conditions may lead to the identification of more new candidate proteins with important functions in lipid metabolism. However, what is known for sure now is that PGs are sitting right at the crossroads of the prenylquinone metabolic pathways.

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