Adv Biochem Engin/Biotechnol (2007) 107: 153–172
DOI 10.1007/10_2007_054

Published online: 31 March 2007

Plastoglobule Lipid Bodies: their Functions in Chloroplasts and their Potential for Applications

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Abstract Plastoglobules are plant lipid bodies localized inside plastids. They have long been considered as mere lipid storage compartments. However, ultrastructural and proteomic data now suggest their involvement in various metabolic pathways, notably the biosynthesis of tocopherols. In this work, the current knowledge on the structure and functions of plastoglobules is reviewed. On the basis of similarities between plastoglobules and seed oleosomes, the potential of plastoglobules for bioengineering applications is discussed.

Keywords Lipid bodies · Plastids · Plastoglobules · Protein purification · Tocopherols

Abbreviations

ABA	Abscissic acid
ADRP	Adipocyte differentiation related protein
AOS	Allene oxide synthase
CCD	Carotenoid cleavage dioxygenase
FBA	Fructose-6-bisphosphate aldolase
JA	Jasmonic acid
NCE	9-*cis*-epoxycarotenoid
OPDA	12-oxo-phytodienoic acid
PAP	Plastid lipid-associated protein
PGL	Plastoglobulin
PQH_2	Plastoquinone
PSII	Photosystem II
ROS	Reactive oxygen species
TAG	Triacylglycerol
VTE	Vitamin E deficient

1 Introduction

Plastids form a group of plant-specific organelles. All plastid types initially derive from proplastids, which are small, undifferentiated organelles abundant in meristematic tissues. As suggested by the etymology of their name ("plassein", the Greek for "to mould" or "shape"), these organelles are highly plastic, both in structure and function. Developmental or environmental stimuli cause plastids to differentiate into specialized plastid types such as photosynthetic chloroplasts, colored chromoplasts or storage plastids (amyloplasts and elaioplasts) [1–3]. Plastids are seen as the result of an ancient endosymbiotic event where a cyanobacterial ancestor invaded a primitive eukaryotic cell [4].

Although plastids are semi-autonomous organelles that retained genetic material as well as transcription and translation machineries, the vast majority of their proteins are encoded in the nucleus and synthesized as cytosolic preproteins with N-terminal transit sequences that need to be imported in plastids (see [5–7] for recent reviews). The transit sequences are recognized by translocon at the outer (Toc) and inner (Tic) chloroplast membranes. The Tic-complex consists of a variety of translocon (Tic20, -21, -22, -40, -110) as well as redox and calcium regulatory components (Tic32, Tic55). Tic22 and Tic110 have been proposed to function as components of the import channel. In addition Tic110, together with Tic40 may function as a co-chaperone recruiting ClpC, cpn60 and, possibly, Hsp70 chaperones to the import site to assist in the folding of the newly imported proteins. The Toc-complex consists of Toc75, a protein conducting channel, and of two homologous GTP-binding proteins, Toc159 and Toc34 functioning as transit sequence receptors exposed at the chloroplast surface. These are encoded by small gene families in Arabidopsis [8]: Toc159 has four homologs in Arabidopsis (AtToc159, -132, -120 and -90) and Toc34 has two

(AtToc34 and -33). Recent genetic and biochemical studies indicate that different combinations of Toc GTPases facilitate the import of specific classes of substrates [6], AtToc159 and AtToc33 together being responsible for the import of constituent proteins of the photosynthetic apparatus.

Although plastids have different shapes and functions, they retain a similar architecture. A double membrane, termed the envelope, delimits the boundary of the organelles. The inside of plastids is composed of an aqueous matrix, the stroma, and in chloroplasts of an extended membrane system, the thylakoids. Lipid bodies, referred to as osmiophilic bodies or plastoglobules, are ubiquitous structures in the stroma. Plastoglobules have been implicated in plant stress response, chloroplast-to-chromoplast transition and thylakoid disassembly in senescing tissues. It is widely accepted that they serve as lipid reservoirs in plastids. Chloroplast plastoglobules have a particular lipid composition and are notably enriched in tocopherols (vitamin E). They are coated with structural proteins from the PAP/PGL/fibrillin family and recent proteomic studies have identified enzymes as genuine plastoglobule components.

Here, we will review the structure, composition and physiological relevance of plastoglobules with a focus on chloroplasts. The potential of plastoglobules for bioengineering applications will then be discussed.

2 Structure and Composition of Plastoglobule Lipid Bodies

Analysis of chloroplast ultrastructure by electron microscopy revealed the presence of lipid bodies ("osmiophilic globuli") in the stroma [9–12]

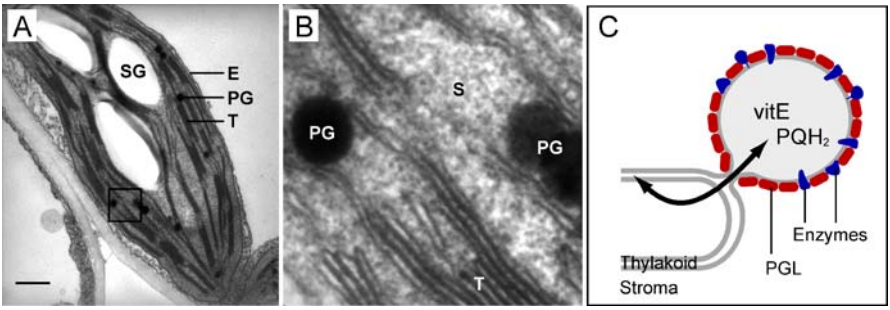

Fig. 1 Structure of plastoglobules. **A** Transmission electron micrograph showing a chloroplast in an Arabidopsis leaf. *Scale bar*: 500 nm. **B** Enlargement from **A**. PG, plastoglobule; SG, starch granule; E, chloroplast envelope membranes; T, thylakoids; S, stroma. **C** Structural model showing the association of a plastoglobule with thylakoid membranes. The half-lipid bilayer surrounding the hydrophobic core of plastoglobule (*light gray*) is continuous with the stroma-side leaflet of thylakoids. Dynamic exchanges of lipids between the compartments are proposed. Structural plastoglobulin proteins (PGL, *red*) as well as enzymes from various metabolic pathways (*blue*) are associated at the periphery of plastoglobules. VitE, vitamin E; PQH_2, plastoquinone

(Fig. 1A,B). The diameter of these bodies, hereafter termed plastoglobules, ranges from 30 nm to 5 μm and varies in different species, plastid types, developmental stages and physiological conditions [13]. In certain species, very large plastoglobules accumulate (e.g. [12, 14]), whereas in others plastoglobule clusters are formed (e.g. [15] as well as personal observations; see Fig. 2A). Although plastoglobule size and abundance are highly variable, the lipid bodies are ubiquitously found in plastids of land plant species, alga [16] and in cyanobacteria [17]. Plastoglobules were proposed to occur in the stroma without connections to other plastid membrane systems [13]. However, only a small portion of chloroplast plastoglobules were liberated from

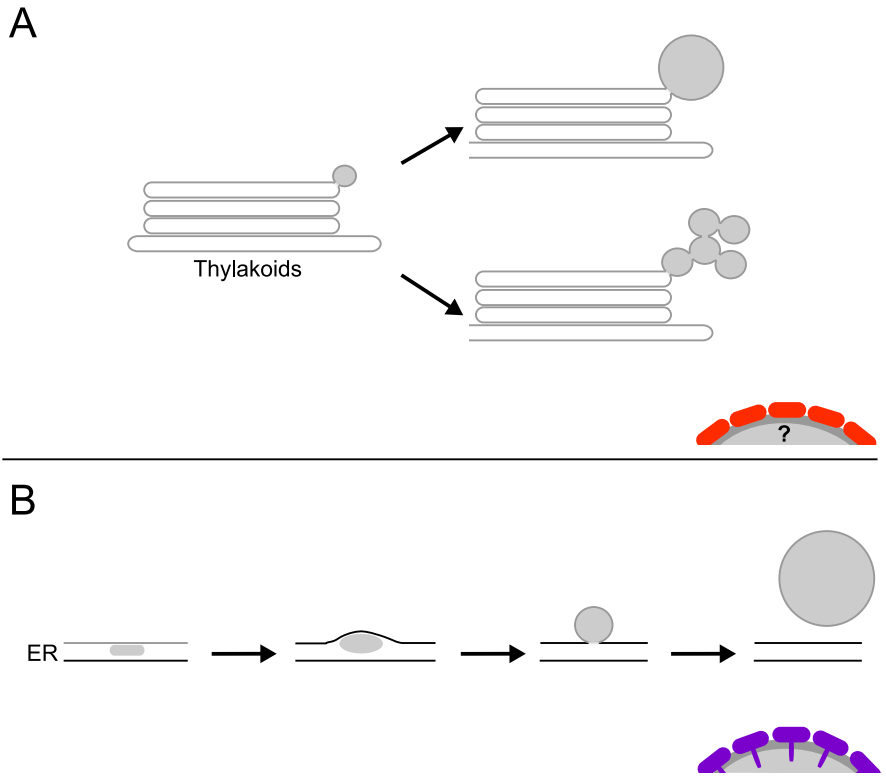

Fig. 2 Models for the formation of plastoglobules (**A**) and oleosomes (**B**). **A** Formation of plastoglobules at the thylakoid membranes occurs through a blistering process. Various developmental and environmental cues including photooxidative stress, senescence and chromoplast differentiation induce enlargement of plastoglobules or the formation of plastoglobule clusters. **B** Oleosome biogenesis in the cytosol. Accumulation of TAGs in sub-domains of the ER precedes budding of oil bodies (after [84]). Oleosins (*purple*) are anchored in oleosomes by a hydrophobic stretch in the manner of a drawing pin, whereas plastoglobulins (*red*) may associate with polar lipids at the periphery of plastoglobules

thylakoid membranes in fractionation experiments [18]. Moreover, recent electron tomographic reconstruction of chloroplasts [15] clearly showed association of plastoglobules with thylakoids, the half-lipid bilayer surrounding the globule forming a continuum with the stroma-side leaflet of thylakoid membranes (see Fig. 1C).

2.1 Lipid Composition of Plastoglobules

The main components of chloroplast plastoglobules are triacylglycerols (TAG) and prenylquinones. Plastoquinone (PQH_2) and tocopherols (vitamin E) are the major prenylquinone constituents while phylloquinone (vitamin K) is present in slight amounts [9, 10, 19, 20, 22]. Traces of chlorophylls and carotenoids (β-carotene, lutein) have also been detected in plastoglobule fractions [9, 10] but have been considered as thylakoid contamination [21]. Similarly, glyco- and phospholipids have been identified in plastoglobule preparations but their genuine association with plastoglobules has been questioned [21]. The lipid composition of plastoglobules from non-green plastids is markedly different from that of chloroplast plastoglobules. Plastoglobules from chloroplasts in senescing leaves, for example, accumulate carotenoid esters, oxidized prenylquinones and free fatty acids [22]. Carotenoid esters are also the major constituents of chromoplast plastoglobules and fibrils [23]. Variations in lipid composition suggest that plastoglobules are highly dynamic structures and that their functions evolve during plastid differentiation.

2.2 Plastoglobulins: Structural Proteins Associated with Plastoglobules

Identification of Plastoglobulins

Early biochemical studies identified nitrogen in purified chloroplast lipid bodies, suggesting the presence of associated proteins [19].

A major peptide of 35 kDa was identified independently by tree groups in bell pepper fruits and designated chromoplast protein B (ChrB, [24]), fibrillin [23] or plastid lipid-associated protein (PAP, [25]). A homologous protein (ChrC) was subsequently identified in cucumber flowers [26]. Expression of fibrillin and ChrC was first proposed to be restricted to chromoplast-containing tissues such as fruits and corollas [23, 27]. However, Pozueta-Romero and colleagues [25] detected fibrillin in leaves and expression of a fibrillin homolog in citrus leaves was reported [28]. Moreover, homologous proteins were identified in leaves from pea (PG1; [29]) and turnip (PAP1-3; [30]), as well as in anthers from rapeseed (BCP32; [31]), indicating that PAP/PGL/fibrillin proteins are not chromoplast-specific and asso-

ciate with various types of plastid lipid bodies. Several cyanobacterial proteins contain a PAP/PGL/fibrillin motif, indicating an ancient origin of the protein family. Related proteins are, however, absent from bacterial, animal and fungal genomes. Since the abbreviations "PAP" and "FIB" already stand for phosphatidic acid phosphatase and fibrillarin, respectively, we proposed to use the term "plastoglobulin" (PGL) to designate proteins from the PAP/PGL/fibrillin family [32].

Localization and Functions of Plastoglobulins

Association of PGLs with plastoglobules has been shown by immunolabeling on chloroplast ultrathin sections [15, 23, 25, 29, 32] and by tagging the proteins with the green fluorescent protein [32]. Several PGLs were also proposed to associate directly with thylakoid membranes [14, 33, 34]. PGLs do not share sequence homology with known enzymes. Moreover, Deruère and collaborators [23] could reconstitute fibrils in vitro by adding purified fibrillin to chromoplast lipids. Overexpressing fibrillin in tobacco lead to an increase in plastoglobule number and to the formation of plastoglobule clusters [14]. The role of PGLs is therefore probably mainly structural, maintaining the shape of the lipid bodies and preventing their coalescence.

Plastoglobulin Gene Families

In different plant species, PGLs form gene families [30, 35]. The Arabidopsis and rice genomes contains 13 and 8 proteins with a PAP/PGL/fibrillin (PF04755) Pfam profile, respectively. Moreover, recent proteomic studies by Ytterberg et al. [36] and Vidi et al. [32] have shown that at least eight different PAP/PGL/fibrillins are associated with Arabidopsis plastoglobules. As revealed by gene chip expression data, Arabidopsis PGLs have distinct expression patterns [37]. It remains to be determined whether the different PGL isoforms have distinct localizations and/or functions.

2.3 Plastoglobules Contain an Assortment of Enzymes

Although PGLs are the most abundant peptides in fibrils and plastoglobules, SDS-PAGE analysis indicated that at least a dozen different proteins associate with plastid lipid bodies [29, 38]. Analysis of the proteome of Arabidopsis plastoglobules [32, 36] revealed enzymes belonging to various biochemical pathways as genuine plastoglobule components (Table 1). Fructose-bis-phosphate aldolase (FBA) isoforms, an epoxycarotenoid dioxygenase (CCD4), the allene oxide synthase (AOS), the tocopherol cyclase (VTE1), putative lipid-modifying enzymes, as well as members of the ABC1/UbiB family were notably identified in both stud-

Table 1 Proteins with structural properties or (predicted) enzymatic activities associated with plastoglobules

AGI code	Gene name or annotation	Functional category[a]	Refs.
At3g26070	Plastoglobulin (AtPGL25/FIB3a)	Structural protein	[27]
At2g42130	Plastoglobulin (AtPGL30/FIB7b)	Structural protein	[27, 31]
At3g23400	Plastoglobulin (AtPGL30.4/FIB4)	Structural protein	[27, 31]
At2g46910	Plastoglobulin (AtPGL31/FIB8)	Structural protein	[31]
At4g22240	Plastoglobulin (AtPGL33/FIB1b)	Structural protein	[27, 31]
At3g58010	Plastoglobulin (AtPGL34/FIB7a)	Structural protein	[27, 31]
At4g04020	Plastoglobulin (AtPGL35/FIB1a)	Structural protein	[27, 31]
At2g35490	Plastoglobulin (AtPGL40/FIB2)	Structural protein	[27, 31]
At2g21330	F6-BiP aldolase (FBA1)	Sugar metabolism	[27, 31]
At4g38970	F6-BiP aldolase (FBA2)	Sugar metabolism	[27, 31]
At2g01140	putative F6-BiP aldolase (FBA3)	Sugar metabolism	[27, 31]
At2g39730	Rubisco activase	Sugar metabolism	[31]
AtCg00490	Rubisco large subunit (RBCL)	Sugar metabolism	[31]
At5g42650	allene oxide synthase (AOS)	Jasmonic acid biosynthesis	[27, 31]
At4g19170	9-*cis*-epoxycarotenoid dioxygenase (CCD4)	Neoxanthin cleavage reaction	[27, 31]
At4g32770	VITAMIN E DEFFICIENT 1 (VTE1)	Tocopherol biosynthesis	[27, 31]
At5g08740	NADH dehydrogenase-like protein, glutathione reductase, Dihydrolipoamide dehydrogenase, FAD-dependent pyridine nucleotide-disulphide	Unknown	[27]
At5g05200[M]	M ABC1 family	*Quinone synthesis*	[27, 31]
At1g79600	ABC1 family	*Quinone synthesis*	[27, 31]
At4g31390	ABC1 family	*Quinone synthesis*	[31]
At1g71810	ABC1 family	*Quinone synthesis*	[31]
At1g54570	esterase/lipase/thioesterase	*Lipid metabolism*	[27, 31]
At3g26840	esterase/lipase/thioesterase	*Lipid metabolism*	[27, 31]
At1g78140[M]	M UbiE methyltransferase-related	*Quinone synthesis*	[27, 31]
At2g41040	UbiE methyltransferase-related	*Quinone synthesis*	[27, 31]
At3g26060	peroxiredoxin Q	Oxidative stress response	[27]
At1g32220	3-β-hydroxysteroid dehydrogenase/isomerase	Unknown	[27, 31]
At2g34460	flavine reductase, steroid biosynthesis	Unknown	[27, 31]
At3g10130	SOUL heme-binding family protein	Unknown	[27, 31]
At1g06690	aldo-keto reductase, ANC transporters family signature	Unknown	[27]
At5g08740	pyridine nucleotide-disulfite oxidoreductase (DhnA-like)	Unknown	[31]

Table 1 (continued)

AGI code	Gene name or annotation	Functional category[a]	Refs.
At1g09340^{O}	NAD-dependent epimerase/ dehydratase, putative RNA binding protein	Unknown	[31]
At3g63140	NAD-dependent epimerase/ dehydratase, putative RNA binding protein	Unknown	[31]
At5g01730^{O}	–	Unknown	[31]
At4g01150	–	Unknown	[31]
At1g28150	–	Unknown	[31]
At1g26090	–	Unknown	[31]
At4g13200	–	Unknown	[27, 31]
At1g52590	–	Unknown	[27]

[a] Italics indicate predicted functions. Sub-cellular localization: AGI codes of proteins for which subcellular localization prediction (TargetP, [103]) was not plastids are labeled with 'M' (mitochondrial prediction) or 'O' (other). AGI code Gene name or annotation Functional category 1

ies. Plastoglobules isolated from red pepper also contained enzymes, namely zeta-carotene desaturase (ZDS), lycopene β-cyclase (LCY-β or CYC-β) and two β-carotene β-hydroxylases (CrtR-β) [36] catalyzing serial reactions in bicyclic carotenoid biosynthesis [39]. These results indicate that plastoglobules in chromoplasts participate in the synthesis of the carotenoids they subsequently sequestrate. The finding indicates that, similarly to cytosolic lipid bodies in yeast or animal cells [40] (see Sect. 4.3), plastoglobules are metabolically active and not mere lipid storage compartments as previously assumed.

3 Proposed Functions of Plastoglobules

3.1 Plastoglobules as Lipid Reservoirs for Thylakoids

The association of plastoglobules with thylakoid membranes [15] suggests that they play a role in thylakoid membrane function, possibly as a reservoir for certain lipids [13]. Indeed, plastoglobules enlarge during thylakoid disassembly in senescing chloroplasts [13, 41–43]. Their accumulation in senescing rosette leaves of Arabidopsis correlates temporally with the activation of diacylglycerol acyltransferase1 (DGAT1) and with enhanced synthesis of

TAGs [44]. Dismantling of thylakoid membranes during senescence allows remobilization of energy for seed production. TAG accumulating in plastoglobules must therefore leave plastids in order to be converted into sugars through β-oxidation and the glyoxylate cycle. Evidence gained from ultrastructural analysis of senescing plastids indicated that plastoglobules are released from gerontoplasts through a blebbing process [42, 45].

3.2 Deposition of Pigments in Chromoplast Plastoglobules

Plastoglobules have also been implicated in chloroplast-to-chromoplast transition. During chromoplast differentiation, plastoglobules enlarge [13] and accumulate esterified carotenoids [21, 23], conferring to fruits and flowers their attractive colors. In certain species, plastoglobules elongate to form fibrillar structures [2, 46].

3.3 Function of Plastoglobules in Plant Stress Response

Morphological Evidence

Enlarged plastoglobules have been described in chloroplasts under conditions resulting in oxidative stress such as drought [14, 33], hypersalinity [47], nitrogen starvation [48], and growth in the presence of heavy metals [49, 50]. In aloe plants exposed to strong sunlight and drought stress, accumulation in leaves of the red carotenoid rhodoxanthin paralleled transformation of chloroplasts into plastoglobule-rich chromoplasts [51]. Studies on spruce and aspen trees have also identified swelling of plastoglobules as part of the physiological response to elevated ozone concentrations [52, 53]. Ageing has also been shown to affect plastoglobule morphology. Older broad bean leaves had significantly larger plastoglobules than younger ones [9]. The same observation was made in rhododendron leaves [54]. Since levels of reactive oxygen species (ROS) are known to rise with time in plastids [55], swelling of plastoglobules in older chloroplasts may again represent a response to the increase of ROS concentration.

Accumulation of Plastoglobulins under Stress Conditions

Up-regulation of several PGLs has been observed as a consequence of various treatments generating photooxidative stress in chloroplasts. These include drought [14, 56–62], cold [60, 62], salt [60, 62], wounding [56, 60], ageing [59] treatment with methyl viologen, [56, 59] and high light stress [14, 57, 59, 60, 63]. Supporting a role of PGLs in stress response, deregulation of these proteins was shown to affect plant growth and stress tolerance.

Overexpression of pepper fibrillin in tobacco enhanced growth under high light intensities as well as drought tolerance [14]. In contrast, antisense potato plants with reduced levels of the PGL C40.4 displayed stunted growth and reduced tuber yield [34]. Recently, Yang et al. [63] showed a correlation between maximal photochemical efficiency of photosystem II (Fv/Fm) and levels of an Arabidopsis PGL in photooxidative stress conditions. Reduction of Fv/Fm values is regarded as photoinhibition of PSII [64]. These results therefore strengthen the view that PGLs directly or indirectly protect the photosynthetic apparatus.

Tocopherol Antioxidants in Plastoglobules

Tocopherols are known to protect membrane lipids from oxidative damage by scavenging radicals and by quenching ROS (reviewed in [65] and [66]). Tocopherols were recently shown to prevent photoinactivation of the PSII [67]. They also protect seed storage lipids from oxidation [68]. Tocopherol synthesis was upregulated after exposure to high light intensities [69, 70] and messenger levels of the tocopherol cyclase (*VTE1*), notably, strongly increased after exposure to strong light [70]. In chloroplasts and cyanobacteria, absence of tocopherols was accompanied by an increased photoinhibition under conditions of photooxidative stress [71, 72]. When reduction of tocopherols and glutathione [70] or zeaxanthin [67] contents were combined, stronger photoinhibition was observed, indicating that photoprotection is guaranteed by a network of antioxidants, including tocopherols. Tocopherols have been detected in all chloroplast membranes and notably in plastoglobules [10, 73]. In these studies, a strong enrichment in prenylquinones was observed in plastoglobules compared to total chloroplast extracts. Moreover, comparison of various plastid types revealed a positive correlation between plastoglobule abundance and prenylquinone contents. Tocopherol measurements in Arabidopsis chloroplast membrane fractions showed that around 50% of the tocopherol pool is localized in plastoglobules, representing a 25-fold enrichment with regard to thylakoids [32].

A Model for the Involvement of Plastoglobules in the Protection of Thylakoids

Changes in plastoglobule morphology and PAP/PGL/fibrillin abundance probably reflect increased needs for antioxidants under stress conditions: Swelling of plastoglobules could indeed allow accumulation of tocopherols (and possibly other antioxidants such as zeaxanthin) and enzymes such as the tocopherol cyclase. A site of action of tocopherols is the thylakoid membrane system. Providing a mechanistic explanation for metabolite trafficking, plastoglobules and thylakoid membranes have been shown to form a continuum [15] potentially allowing tocopherols to diffuse be-

tween these two compartments (Fig. 1C). Phylloquinone and PQH_2, which were also measured in plastoglobules, are both involved in the electron transport chain in thylakoid membranes. Like tocopherols, other prenyl quinones probably extensively diffuse in the thylakoid-plastoglobule lipid continuum.

Under photooxidative stress conditions, charge separation at the PSII occurs faster than oxidation of PSII acceptors [74], which leads to photoinhibition. Plastoglobules may therefore represent a compartment to sequestrate reduced quinone pools. Furthermore, the presence of tocopherols in plastoglobules may protect this pool from oxidative damage. Similarly, tocopherols accumulating in chromoplasts plastoglobules and fibrils [75] probably protect carotenoids from oxidation.

Plastoglobules and Signaling Networks

Abscissic acid (ABA) is known to mediate many aspects of plant adaptation to abiotic stresses [76]. ABA was shown to induce the expression of *CDSP34*, a potato gene highly similar to fibrillin and Arabidopsis *PGL35* [57]. The authors further showed that transcripts from the tomato *CDSP34* ortholog also accumulated after either ABA or dehydration treatments in wild-type plants and in *flacca* tomato mutants (impaired in ABA biosynthesis). In contrast, accumulation of the CDSP34 protein did not occur in the *flacca* background unless exogenous ABA was supplied, indicating that an ABA-dependant post-transcriptional mechanism controls the expression of *CDSP34*. In a recent report, Yang et al. [63] demonstrated accumulation of PGL35 after exogenous application of ABA. Moreover, using yeast two-hybrid and pull-down assays, a direct interaction between the transit peptide of PGL35 and ABI1 (a key player of ABA signaling) was shown, further implicating ABA in the post-transcriptional regulation of PGL expression [63]. *VTE1* transcripts also accumulated after 3 h treatment with 10 μM ABA (3.3-fold, Genevestigator, [37]). These observations suggest that ABA induces the accumulation of tocopherols both by up-regulating their synthesis and by increasing the volume of plastoglobules, their storage compartment.

The first, rate-limiting step of ABA biosynthesis is the cleavage of 9-*cis*-epoxycarotenoids (NCE) [77]. Interestingly, a NCE dioxygenase isoform (CCD4, At4g19170) was identified in the plastoglobule proteome [32, 36]. To address the possible involvement of CCD4 in ABA biosynthesis, future work will address whether the protein possesses a NCE cleavage activity. Arabidopsis *ccd4* null mutants may also prove useful tools to answer this question. Plastoglobules serve as storage compartments for quinones and tocopherols in chloroplasts. They might therefore represent "sensors" of the redox status of plastids. Taking this into consideration, the possible localization of (part of) ABA biosynthesis in plastoglobules may be rationalized. The allene oxide

synthase, involved in the biosynthesis of jasmonic acid (JA) precursor OPDA, was identified in the plastoglobule proteome [32, 36]. As for ABA biosynthesis, plastoglobules in addition to containing lipid precursors may act as redox sensors for JA biosynthesis.

4
Common Features of Lipid Bodies in Plant, Fungal and Animal Cells

In plants, animals and microorganisms, lipid bodies have functions as diverse as energy storage, structural lipid storage, lipid transport and lipid metabolism (reviewed in [40]). However, common themes exist between lipid bodies which will be briefly discussed.

4.1
General Organization of Lipid Bodies

Lipid bodies consist of a hydrophobic core surrounded by a monolayer of amphiphatic lipids. Peripherally associated proteins are found in most types of lipid bodies and are more or less tightly bound to their surface. Such an architecture was proposed for chromoplast fibrils [23, 78], and chloroplast plastoglobules [29].

4.2
Association of Peripheral Proteins

Proteins associated with lipid bodies have diverse physicochemical properties and topologies, reflecting various modes of association with the lipidic structures. Hydrophobic sequences in several peripheral oil body proteins ensure their association with the lipids. For example, hydrophobic domains in perilipin were shown to be essential for targeting and anchoring the protein to lipid droplets in adipocytes [79]. In desiccation tolerant seeds, oil bodies are coated with oleosins. A central hydrophobic domain in the proteins, referred to as "proline knot motif", is essential for their association with oil bodies [80] (Fig. 2B). Interestingly, association of the hepatitis C virus core protein with cytosolic lipid droplets in mammalian cells requires a proline-containing domain similar to that of oleosins [81].

Although several lipid body proteins are characterized by hydrophobic domains, others lack large apolar regions. The sequence of adipocyte differentiation related protein (ADRP), for example, does not contain obvious lipid-binding motifs (hydrophobic domains or amphiphatic α-helices; [40]) and discontinuous stretches of the protein are necessary for targeting to lipid bodies [82]. PGL proteins also lack strongly hydrophobic domains and their association with plastoglobules may therefore rely on interactions with sur-

face lipids [30]. Indeed, several PGLs were identified in 8 M urea chloroplast membrane extractions [83], consistent with peripheral association with plastoglobules.

Lipid body proteins with highly diverse properties have similar functions. They are generally thought to prevent coalescence of lipid bodies with neighboring lipophilic structures. Oil body coalescence was for instance observed in seeds from oleosin-deficient plants [84]. In addition, certain lipid body proteins including ADRP induce the formation and regulate the size of the lipid bodies [85, 86]. Several lines of evidence (see Sect. 2.2) suggest that PGLs may also be involved in regulating the morphology of plastid lipid bodies. However, future studies are needed to address the underlying molecular mechanisms.

4.3 Metabolic Functions

The identification of enzymes associated with plastoglobules indicates that chloroplast lipid bodies are metabolically active [32, 36]. Moreover, the observation that chloroplast, chromoplast and etioplast low-density fractions have different protein assortments [36] suggests that enzymatic functions of plastid lipid bodies are highly dynamic.

The analysis of the proteome from Chinese hamster ovary (CHO) K2 cell lipid droplets also revealed metabolic functions [87]. In addition to structural proteins (including ADRP), enzymes involved in the synthesis, storage, utilization, and degradation of cholesterol esters and TAGs were identified. Rab GTPases, as well as a GTPase activating protein (p50RhoGAP) were also detected, suggesting functions in membrane traffic and signaling. Enzymes in lipid metabolism were also shown to associate with yeast oil bodies [88, 89]. Interestingly, sterol-Δ^{24}-methyltransferase (Erg6), but not other enzymes from ergosterol biosynthesis was localized to lipid bodies, indicating shuttling of intermediates between the endoplasmic reticulum and the lipid bodies [40]. Tocopherol biosynthesis in plastids represents a similar situation, with VTE1 associated with plastoglobules and the other metabolic steps occurring at the envelope [90]. With the exception of a 11-β-hydroxysteroid dehydrogenase-like protein, a calcium binding protein (ATS1), as well as two proteins of unknown function, proteins identified in association with oleosomes were structural oleosins [91]. Analysis of the *Brassica napus* seed proteome identified in addition a putative short chain dehydrogenase/reductase and a protein similar to GDSL-motif lipase/hydrolases, suggesting functions in lipid metabolism [92]. In the studies by Jolivet et al. [91] and Katavic et al. [92], mature oilseeds were used for protein identification. Because the protein assortment of oleosomes may be dynamic, it would however be interesting to analyze oil body proteomes in germinating and maturing seeds.

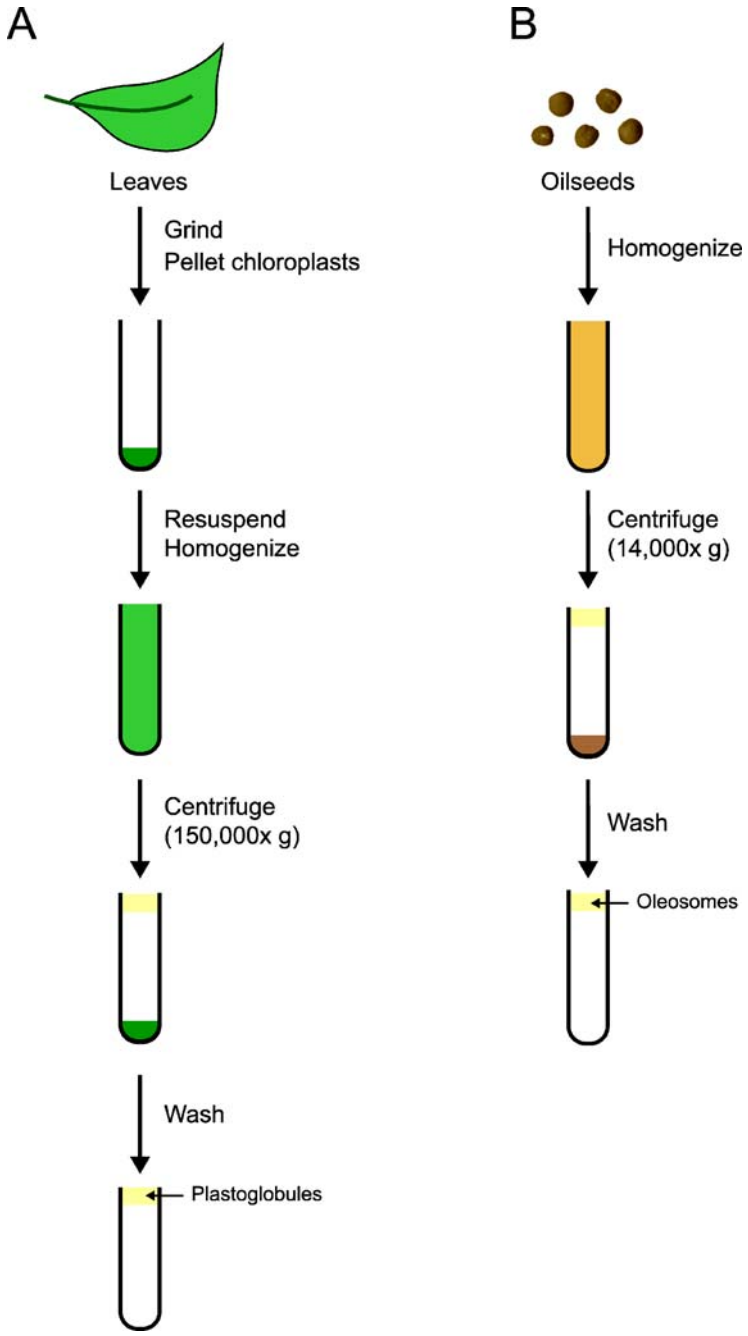

Fig. 3 Procedures for the purification of plant oil bodies. Purification schemes for chloroplast plastoglobules **A** adapted from [36] and oilseed oleosomes **B** as described in [95]. Both procedures rely on the low density of the lipid bodies

5
Potential of Plastoglobules for Bioengineering Applications

5.1
Purification of Plant Lipid Bodies

Purification procedures for plastoglobules [29, 32, 36, 93] and oleosomes [94, 95] rely on the low density of the lipid bodies (Fig. 3). In both systems, extracts containing plastoglobules or oleosomes are centrifuged. Membrane systems such as thylakoid or ER which contain high protein/lipid ratios sediment, while low-density lipid bodies accumulate on top of the supernatant and are recovered. While sucrose density gradients have been used in several studies to isolate plastoglobules [29, 32, 38, 93], Ytterberg et al. [36] have shown that a simpler procedure, similar to that used by van Rooijen et al. [95] for oleosome extraction, was also adequate for plastoglobule purification.

5.2
Plant Lipid Bodies as Purification Matrices for Recombinant Proteins

To date, proteins used in medicine as diagnostic reagents, drugs or vaccines are mostly produced by microbial or animal cell fermentation. These manufacturing systems allow highly controlled procedures but have disadvantages in term of cost and scalability. Pathogen contamination of animal cell cultures also represent an important safety issue. Plants stand as alternative systems for the production of recombinant proteins at lower costs ("molecular farming") [96, 97]. They allow large-scale production with accurate folding and assembly of protein complexes [98–101].

Important issues for industrial production of plant-derived recombinant proteins are extraction and purification [102]. Standard protocols include homogenization of plant biomass followed by chromatographic methods. However, developing cost-effective preliminary (or alternative) purification steps is of great interest since the high abundance of secondary compounds, especially in tobacco, is problematic for chromatographic procedures [97]. Plant cells contain roughly 30 000 different proteins whereas only nine different proteins were identified in oleosomes [91] and the proteome of plastoglobules consists solely of about 20 core components [32, 36]. Targeting recombinant proteins to lipid bodies and subsequent isolation of lipid bodies therefore represents an effective purification step.

The use of oleosomes as carriers for recombinant proteins was proposed by van Rooijen et al. [103] and the system is now being developed by SemBioSys (http://www.sembiosys.com/Index.aspx; [104]). The oil body-oleosin (Stratosome) system has for instance been applied to the production of biologically active human insulin [105]. A similar approach may be followed

using plastoglobules derived from leaf crops such as tobacco. It would combine the high potential of plastids for recombinant protein accumulation [106, 107] with simple purification procedures. Moreover, sequestration of foreign proteins in lipid bodies may limit deleterious effects on chloroplast metabolism.

5.3
Plastoglobules as Sources of Tocopherol

Vitamin E is widely used in the industry, notably as a dietary supplement for animal nutrition as well as antioxidant in cosmetic and food preparations: about 40 000 tons of tocopherols were produced in 2002 [108]. Most tocopherol used in industrial applications is chemically produced. However, plants are interesting sources of tocopherol since (i) natural α-tocopherol has 1.5 more vitamin E activity than its synthetic racemic counterpart [66]. (ii) Plants represent renewable carbon sources, in contrast to fossil oils used for the chemical synthesis of tocopherol acetate. (iii) Additives from natural sources are becoming popular for consumers. To date, natural vitamin E is derived from soybean oil [108]. Considering the high tocopherol content of plastoglobules [32], it will be interesting to address the potential of plastoglobules as sources of tocopherols. Vitamin E could for example represent a valuable by-product in molecular farming applications.

6
Outlook

Plastoglobules have been observed in various plastid types for more than 40 years but have attracted little attention until recently. The conception of plastoglobules being passive storage compartments is now shifting toward a much more complex and dynamic view. The emerging picture implies plastoglobules in biosynthetic processes and in the ABA and JA signaling networks. The recent data on plastoglobules need to be connected with studies on other lipid bodies in yeast and animal systems where the same paradigm shift toward dynamic and metabolically active structures occurs. The publications reviewed in this work lay down a molecular basis for plastoglobule function, notably in stress protection but also raise many new questions: How are biosynthetic intermediates trafficked between plastoglobules and the other chloroplast membrane systems? How do plastoglobules integrate in ABA (and JA) regulatory networks? What is the function of the "unknown" plastoglobule proteins?

Evidence in the literature indicates a dynamic lipid composition of plastoglobules. The data are, however, scarce and better characterization of plastoglobule lipid composition in various developmental stages or environmen-

tal conditions will help to understand their physiological functions and may identify interesting compounds.

In the absence of structural information on PGLs, hypotheses were suggested regarding their mode of association with plastoglobules. Getting a better understanding of the mechanisms underlying protein targeting to plastoglobules may prompt new applications. We have drawn here the parallel between plastoglobules-PGLs and seed oil body-oleosin systems. Future work will address the potential of plastoglobules as targeting destinations for recombinant proteins. Also, simplification of the plastoglobule purification scheme should be considered, in order notably to reduce centrifugation steps. The identification of optimal conditions where plastoglobules strongly accumulate in plastids (e.g. upon stress treatment or during the ageing process) should also be part of future investigations.

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