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The Plastoglobule: A Bag Full of Lipid Biochemistry Tricks†

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ABSTRACT

Plastoglobules are lipoprotein particles contained in chloroplasts and other plastids. They have long been regarded as lipid storage droplets. New results now indicate that plastoglobules actively participate in prenylquinone and other metabolic pathways. Structural work shows physical attachment of plastoglobules to the thylakoid membrane probably enabling the exchange of lipid molecules between the membrane compartments. This review will give a summary of research, past and present, attempting to elucidate the role of plastoglobules in the context of plastid function.

INTRODUCTION

The chloroplast, the green organelle of plants, hosts photosynthesis and many biosynthetic pathways. Based on structural and biochemical comparisons as well as phylogenetic analyses, it is generally accepted that chloroplasts and other plastid types originate from cyanobacteria. In a process referred to as endosymbiosis the prokaryote became the eukaryotic organelle. Endosymbiosis involves the transfer of the majority of the genetic material of the cyanobacterial chloroplast precursor to the host cell nucleus (1–3). The nuclear-encoded chloroplast proteins acquired targeting sequences (transit sequences) allowing for their import into the organelle. By importing specific sets of proteins, higher plants obtained the capacity to produce highly specialized plastid types such as amyloplasts and chromoplasts.

Plastids (Fig. 1) are delimited by a double membrane envelope controlling the exchange of metabolites with the cytosol as well as the import of nuclear-encoded proteins. The thylakoids, the internal photosynthetic membrane system, are contained in the soluble phase, the stroma. Chloroplasts also contain a rudimentary genetic apparatus and protein synthesis machinery. The primary photosynthetic assimilate is deposited in starch granules. In addition to these aforementioned structural elements the chloroplasts also contain plastoglobules (4). Generally the chloroplast and its functions have been well described, but the plastoglobules have remained a white spot on the organellar map. Plastoglobules, at first termed ''osmiophilic globules'' (5), are low density lipoprotein bodies, with an average diameter of 50–100 nm in vegetative leaf cells. Plastoglobules consist of an outer polar lipid monolayer containing neutral lipids such as prenylquinones, carotenoids and others. In addition to the lipid components plastoglobules were known to harbor protein (Fig. 2) (6,7). Plastoglobule dimensions may vary from 30 nm to several micrometers (8) depending on developmental stage and environmental conditions. A series of studies have reported an increase in plastoglobule size and number under biotic and abiotic stress conditions. These findings suggested an implication of plastoglobules in plant stress response. Nevertheless, plastoglobules have widely been considered passive lipid storage droplets.

Historically, the plastoglobule protein discovered first was fibrillin. Fibrillin coats the plastoglobule-related carotenoid-containing color fibrils in red pepper chromoplasts (9). These fibrils are known to originate from plastoglobules and are structurally related (10). Later, fibrillin and its homologs were also demonstrated to associate with plastoglobules in leaf tissue and also termed plastid-lipid associated proteins (PAP) or plastoglobulins (11,12). For some time the fibrillins/PAPs/plastoglobulins remained the only known plastoglobule components. Due to their common localization at plastoglobules or related structures (13), ''plastoglobulin'' appears the most appropriate name to qualify the members of this protein family.

In an effort to discover the composition and functions of plastoglobules, the Arabidopsis plastoglobule proteome has recently been determined (14,15). A total of 34 candidate plastoglobule proteins were present in the proteome. The 34 proteins form three groups: plastoglobulins, known metabolic enzymes and proteins of unknown function. Eight of the 13 homologs constituting the Arabidopsis plastoglobulin family (16) were present in the plastoglobule proteome. The six known enzymes include the three isoforms of fructose-bisphosphate-aldolase, the allene oxide synthase involved in jasmonate biosynthesis, the tocopherol cyclase AtVTE1 and the carotenoid cleavage dioxygenase AtCCD4. The proteins of unknown function consist largely of predicted enzymes presumably involved in lipid metabolism. In addition to the three groups, known thylakoid proteins were also present in the proteome but were not considered to be bona fide plastoglobule proteins.

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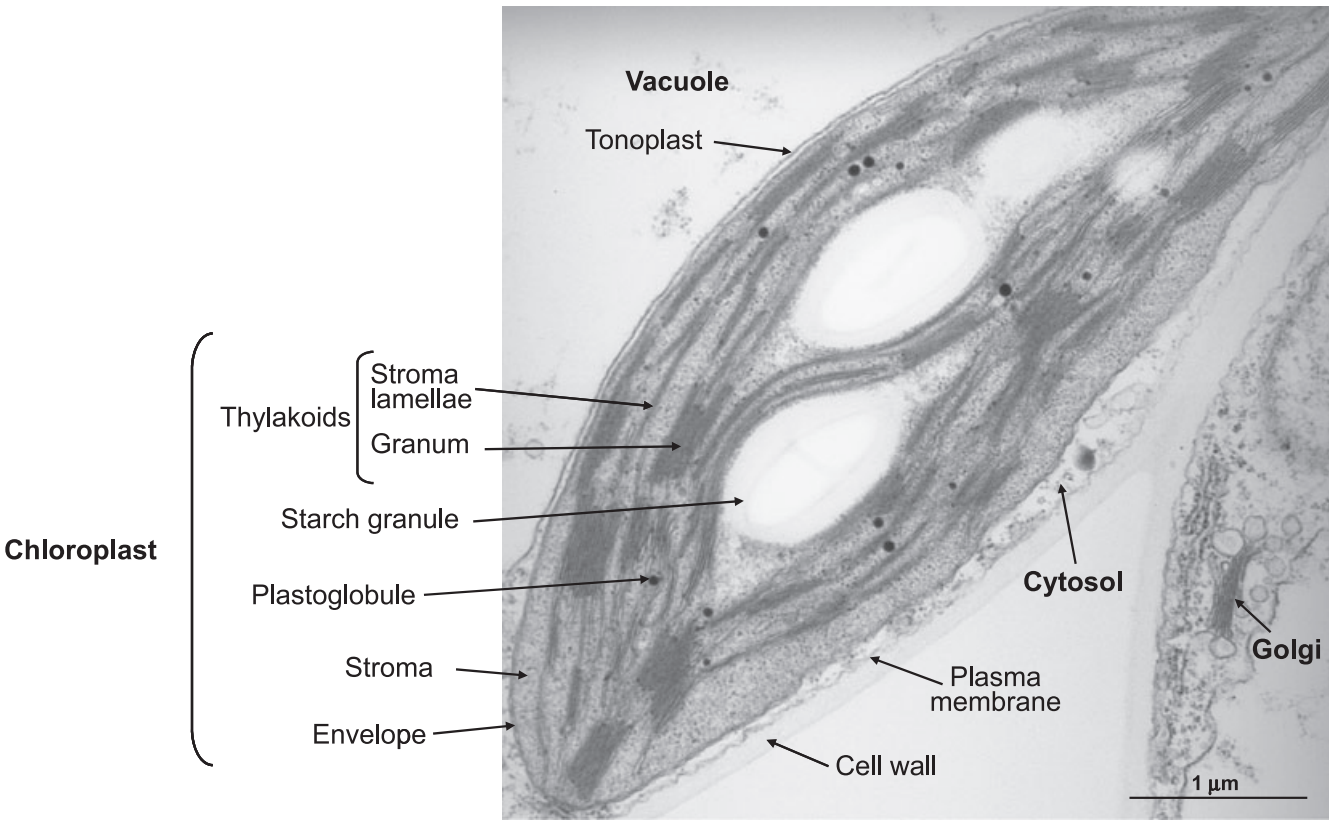

Figure 1. Electron micrograph of an Arabidopsis cell. Chloroplasts are composed of the dual membrane envelope, thylakoids, stroma, starch granules and plastoglobules. Diverse compartments of the plant cell are indicated in bold lettering.

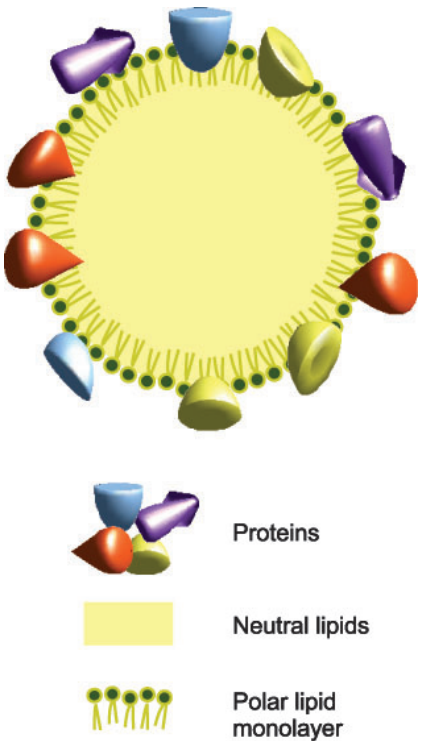

Figure 2. Model of plastoglobule structure. Plastoglobules are composed of neutral lipids surrounded by a polar lipid monolayer and coated with proteins.

In summary, the protein composition suggests that plastoglobules function in diverse metabolic pathways. Importantly, the data indicate that plastoglobules do not simply store lipids but actively participate in their metabolism. Interestingly, metabolic activity in lipoprotein particles is an emerging general concept in a move away from the more static storage models (17–19).

PLASTOGLOBULES PARTICIPATE IN STRESS RESPONSES

Plastoglobules contain a large proportion of an important prenylquinone compound, α-tocopherol (also known as vitamin E) (14). The tocopherols protect plastid membrane lipids against peroxidative damage and Photosystem II against photoinhibition (20). Moreover, the presence of tocopherol also suggests a role for plastoglobules in protecting the thylakoid membrane against oxidative stress. Most of the enzymatic activities of the tocopherol biosynthetic pathway were localized to the inner membrane of the chloroplast envelope. However, the tocopherol cyclase (VTE1, for Vitamin E defective) catalyzing the conversion of 2,3-dimethyl-5-phytyl-1,4-hydroquinol into γ-tocopherol was identified in the plastoglobule proteome (14,15). Several lines of evidence show that VTE1 is specifically attached to plastoglobules and may be completely absent from envelope membranes (14,21). Thus, the available data suggest that the tocopherol biosynthesis pathway passes through plastoglobules. In agreement with metabolic activity, plastoglobules may thus not just

store this prenylquinone compound but participate in its synthesis.

In 1971, Taylor and Craig (22) reported that "the number of osmiophilic droplets (...) appeared to increase in chloroplasts of the upper mesophyll" in Sorghum after 3 days of 12 h light at 25°C/12 h dark at 10°C, compared to 25°C day/night controls. Hall *et al*. (23) observed that "large osmiophilic globules are present" in calcium- or magnesium-deficient maize plants, while in sulfur-deficient plants "Osmiophilic globules (...) appear to be smaller and more numerous than in chloroplasts from the full-nutrient plants." Exposure to heavy metals such as cadmium has been shown to increase the size and number of plastoglobules in a number of species such as pea (24) or the Cd-hyperaccumulating *Sedum alfredii* ecotype Hance (25). Size and number of plastoglobules also respond to osmotic stress: in drought-stressed potato plants, plastoglobules are bigger and more numerous than in well-watered ones (26); a salt-adapted tobacco cell line grown in the presence of 429 mM NaCl accumulated plastoglobules of different sizes (27). As in most of these examples, changes or reduction in the thylakoid system also took place, it is tempting to hypothesize that the increase in size and number of plastoglobules is linked to the accumulation of products from thylakoid catabolism. Under high light stress or nitrogen starvation, size and number of plastoglobules also increase (23,28). Under such abiotic stress as well as during senescence, a large part of the chlorophyll is degraded, resulting in the release of phytol and chlorophyllide. Chlorophyllide which has phototoxic properties is rapidly catabolized (for a review, see Hortensteiner [29]). Two recent studies (28,30) demonstrated that part of the released phytol, considered potentially toxic due to its detergent-like properties, is incorporated into fatty acid phytyl esters (FAPEs). A significant proportion of the FAPEs accumulate in plastoglobules (28). This finding validates the concept of accumulation of thylakoid catabolites in plastoglobules. In this case, plastoglobules may provide a means to protect thylakoid membranes from free phytol by esterification and FAPE sequestration.

Additional evidence for the implication of plastoglobules in plant abiotic stress responses stems from the study of the characteristic plastoglobule proteins: the plastoglobulins.

PLASTOGLOBULINS AND THEIR POTENTIAL FUNCTIONS

Immunogold electron microscopy experiments located plastoglobulins at the chloroplast plastoglobule and pepper chromoplast fibril perimeters, respectively (9,11,12,14,21). When incubating purified bell pepper fibrillin (the prototypical plastoglobulin) with polar and isoprenoid lipids (carotenoids), reconstituted color fibrils formed (9). Similarly, while the pepper plastoglobulin has been shown in tobacco to partition between thylakoids and stroma, the overproduction of the protein increases the number of plastoglobules per chloroplast *in planta* (31). These results suggest a role for plastoglobulins in the formation and maintenance of fibril as well as plastoglobule structure.

Proteomic studies demonstrate an implication of plastoglobulins in stress responses. A plastoglobulin has been identified having one of the 12 "low abundant" proteins that are significantly induced by cold treatment in rice leaves (32). Furthermore, the plastoglobulin AtPGL30.4 (At3g23400) has also been identified among five of the Arabidopsis proteins phosphorylated during the defense response to *Pseudomonas syringae* pv. tomato DC3000 (33). A comparative proteome analysis has shown that four members of the Arabidopsis plastoglobulin family accumulate in response to a 1 to 5 day high-intensity light treatment (34).

The mRNA expression patterns of plastoglobulin homologs also support their implication in resistance to stress. For instance, transcript of a bell pepper plastoglobulin accumulates within 24 h after mechanical wounding and in drought-stressed leaves in a light-dependent way (35). The promoter of this plastoglobulin is induced by wounding, drought, salt or oxidant stresses under light but not dark conditions, suggesting an induction by photo-oxidative stress, putatively *via* reactive oxygen species production by photosystems (35,36). In potato, the plastoglobulin CDSP34 (Chloroplastic Drought-induced Stress Protein 34) was identified as one of the proteins accumulating during the early stages of drought stress, and later found to be induced by high-light and various oxidative stresses (37–39). Abscisic acid (ABA), a plant hormone responsible for the integration of several stress responses (40), induces the expression of at least some of the plastoglobulins (37,41). Furthermore, Yang *et al*. (41) demonstrated that the expression of the Arabidopsis plastoglobulin AtPGL35 (At4g04020) is regulated by ABA response regulators ABI1 and ABI2. As described above, under such stress conditions plastoglobule size and/or number generally increase. Thus, the maintenance of plastoglobule structure and the regulation of their size and number is probably linked to the structural action of plastoglobulins that accumulate under these stress conditions.

Plastoglobulin C40.4 was identified as a transcript strongly upregulated in leaves of tuberizing *Solanum demissum* (42). Antisense plants with reduced level of C40.4 transcript showed retarded growth and reduced tuberization rate compared with wild-type plants. These results suggest that plastoglobulins participate, directly or indirectly, in a developmental regulatory mechanism. Such an implication has also been documented by Rey *et al*. (31): transgenic tobacco plants with higher level of pepper plastoglobulin proteins exhibit accelerated floral development and enhanced growth under high light conditions, and better growth rate than wild-type plants after 9 days of drought treatment. The mode of action of these plastoglobulins on development still needs to be elucidated, but it has been suggested that the proteins exert a protective effect on photosystems or thylakoid membranes, leading to better photosynthetic efficiency (42).

PLASTOGLOBULES ARE PHYSICALLY AND FUNCTIONALLY LINKED TO THYLAKOIDS

Plastoglobules are often observed in close proximity to thylakoids in electron micrographs, mostly localized at the highly curved thylakoid margins. The location of plastoglobules at the curves of the thylakoid margins may favor their formation—or "blistering." Using electron tomography, Austin *et al*. (21) obtained detailed insight into the ultrastructure of plastoglobules. The tomographic study demonstrates that the outer leaflet of the lipid bilayer of thylakoids is contiguous with the polar lipid half-bilayer surrounding plastoglobules

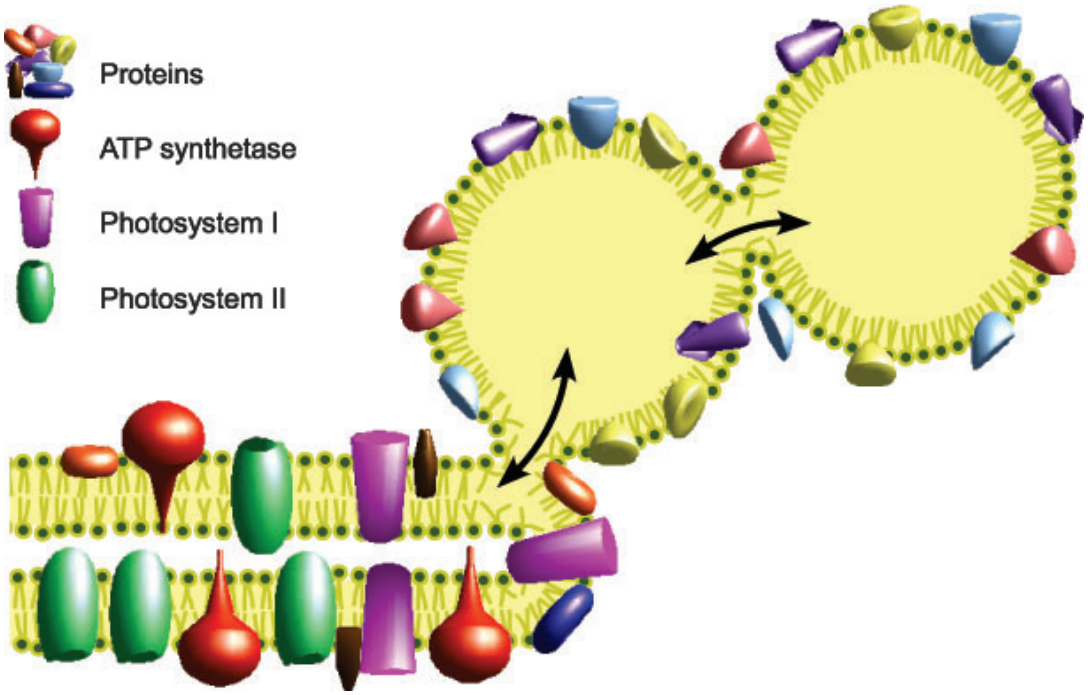

Figure 3. Model of coupling of plastoglobules to thylakoids. The physical link between plastoglobules and thylakoids may allow the bidirectional channeling (double arrows) of lipid metabolites.

(Fig. 3). Plastoglobules may form extensive networks: clusters of plastoglobules not directly connected to thylakoids but linked to other plastoglobules in a kinked configuration. These observations support a model in which plastoglobules are physically coupled to thylakoids in a way possibly allowing the bidirectional channeling of lipid metabolites (Fig. 3).

Plastoglobules and thylakoids are not only structurally but also functionally linked. This is evident in their sharing of metabolites such as tocopherol or FAPEs (see above). Lohmann *et al*. (43) recently analyzed the distribution in chloroplast membranes of phylloquinone (vitamin K1), a prenylquinone that participates in the photosynthetic electron transfer chain of Photosystem I. Two molecules of phylloquinone are present at each Photosystem I complex. However, the overall stoichiometry has been estimated at 3 mol of phylloquinone for 1 mol of Photosystem I. This estimation suggests that phylloquinone is not exclusively associated with Photosystem I but exists in an additional pool. Part of the phylloquinone pool is localized at the plastoglobules (43,44). Additional evidence for a function of plastoglobules in phylloquinone metabolism stems from the analysis of the Arabidopsis methyl transferase *menG* mutant. Much of 2-phytyl-1,4-naphthoquinone, the precursor of phylloquinone accumulated in the *menG* mutant, is present in plastoglobules. The MenG enzyme, responsible for the conversion of 2-phytyl-1,4-naphthoquinone into phylloquinone, was characterized in the same study (43). Interestingly, the transient production of AtMenG-GFP fusions in Arabidopsis leaves resulted in a spotty fluorescence pattern resembling that of plastoglobule localized GFP-fusion proteins. While AtMenG was not present in the plastoglobule proteome, we speculate it may be a loosely associated component which is lost during the purification procedure. We therefore hypothesize that the precursor of phylloquinone may not only accumulate but may also be converted to phylloquinone in plastoglobules. Phylloquinone may then diffuse to Photosystem I through the physical connection between plastoglobules and thylakoids, while any excess remains in plastoglobules. Photosystem I preferentially localizes to the appressed granal domains, that is stroma lamellae, grana endmembranes and grana margins (45,46). Thus the colocalization of Photosystem I and plastoglobules at thylakoid margins may facilitate distribution of phylloquinone between the two membrane compartments.

Plastoquinone (PQ-9) is a small diffusible electron carrier that participates in the photosynthetic electron transfer chain between Photosystem II and the cytochrome b6/f complex. Two molecules of PQ-9 are present at the Q_A and Q_B sites of Photosystem II, respectively. In addition, mobile plastoquinone molecules, forming the plastoquinone pool, are present in thylakoid membranes and electronically couple Photosystem II to the cytochrome b6/f complex (47,48). The presence of plastoquinone in plastoglobules has been reported earlier (44,49,50) and suggests that a part of the pool may disengage from photosynthetic electron transfer and relocate to the lipid particles. However, the exact distribution of plastoquinone between plastid membranes remains to be determined.

PLASTOGLOBULES ARE INVOLVED IN CHROMOPLASTOGENESIS AND SENESCENCE

During senescence plastoglobule size and number increase while thylakoid membranes disintegrate and disappear. Presumably, plastoglobules accumulate some of the catabolites originating from thylakoid degradation (51–55). Plastoglobules

reportedly contain triacylglycerol (TAG) but little galactolipid (28,50). It has been proposed that TAG accumulating in plastoglobules originates from thylakoid galactolipid mobilization and represents an intermediate step in the conversion of thylakoid fatty acids to phloem-mobile sucrose (52). Padham *et al*. (56) identified a putative TAG lipase that could mobilize the TAG accumulated in plastoglobules. By confocal microscopy the putative TAG lipase was shown to colocalize with neutral lipids suggesting its localization at the plastoglobule. Antisense Arabidopsis plants with reduced content of TAG lipase exhibit stunted growth and a delay in the onset of senescence in rosette leaves. In addition, chloroplasts of these transgenic plants have fewer and deformed thylakoids and smaller but more numerous plastoglobules. The delay in rosette senescence suggests that the deformed thylakoids are loose but not yet disintegrating. Therefore, the repression of the TAG lipase may delay the initiation of a senescence program, thus explaining the absence of enlarged plastoglobules in these deformed but not senescent chloroplasts.

Plastoglobulins and plastoglobules are also believed to participate in carotenoid sequestration (6,50). Indeed, screening for proteins mainly present in chromoplasts led to the identification of plastoglobulins/PAP/fibrillins in cucumber flowers (57) and bell pepper fruits (9,12). Furthermore, a proteomic study (58) identified a plastoglobulin as one of the most abundant proteins in bell pepper chromoplast. The implication of plastoglobulins in carotenoid sequestration and chromoplast genesis has recently been further demonstrated by two independent studies. Suppression of the plastoglobulin *LeCHRC* expression by RNAi leads to a 30% reduction in carotenoid level per unit protein in tomato flowers (59). However, the carotenoid level accumulation in leaves of these transgenic plants was similar to the one in wild-type plants. This suggests that plastoglobulin LeCHRC is implicated in accumulation of chromoplast carotenoids essential to color flowers and fruits, but not of chloroplast carotenoids involved in light-harvesting antennae or dissipation of excess light energy (60). Moreover, it is interesting to note that the down-regulated tomato plants exhibit higher susceptibility to *Botrytis cinerea* than wild-type plants, highlighting an additional role for this plastoglobulin in biotic stress response.

In a complementary approach, Simkin *et al*. (61) showed that the overexpression of a pepper plastoglobulin in tomato plant induces an increase in carotenoid and carotenoid-derived volatile content of ripening fruits. Furthermore, the overexpressed plastoglobulin appears to delay thylakoid disintegration during chloroplast to chromoplast transition, leading to the formation of transient plastids with two co-existing zones: one with chloroplast characteristics (*i.e*. well-developed thylakoid membranes) and the other with chromoplast ones (*i.e*. carotenoid crystals and enlarged plastoglobules), suggesting a preventive role of this plastoglobulin in thylakoid dismantlement during chromoplastogenesis.

Such an intermediate plastid has also been described in a *Capsicum annuum stay-green* mutant (62). In *stay-green* mutants, senescence is delayed and chlorophyll is retained. In carotenogenic *stay-green* mutant fruits, the maintenance of chlorophyll and thylakoid membranes coexists with the *de novo* biosynthesis of carotenoids. These carotenoids accumulate in plastoglobules, inducing an increase in size and number of plastoglobules while the thylakoids remain.

Table 1. Lipids reported to localize to plastoglobules.

Lipids	References
Prenylquinones	
Tocopherol	(14)
DMPQ	(14)
Phylloquinone (vitamin K1)	(43)
Carotenoids	(6,7,9)
Fatty acid phythyl esters	(28)
Plastoquinone	(44,49,50)
Triacylglycerol	(28,50,52)

DMPQ = 2,3-dimethyl-5-phytyl-1,4-hydroquinol.

In the plastoglobule proteome, AtCCD4, one of the nine members of the Arabidopsis carotenoid cleavage dioxygenase (CCD) family has been identified (14,15). CCDs cleave specific double bonds of diverse carotenoid substrates and are involved either in ABA biosynthesis or in carotenoid catabolism (63,64). Such a role for AtCCD4 would be in accordance with the implication of plastoglobules in stress response and in carotenoid sequestration. The enzymatic activity of AtCCD4 has not been characterized until now. However, CmCCD4a, a Chrysanthemum homolog of AtCCD4, has been shown to be responsible for the white color of petals (65). The authors propose that CmCCD4a catalyzes carotenoid degradation which causes the disappearance of yellow and the emergence of white color. Recently a saffron (*Crocus sativus*) carotenoid dioxygenase, CsCCD4a, was localized to plastoglobules and implicated in the formation and release of β-ionone from stigma tissue (66). The divergent functions of *CmCCD4a* and *CsCCD4a* suggest that the CCD4 homologs have varying physiological roles depending on species and tissue.

In plastoglobules from red pepper chromoplasts, Ytterberg *et al*. (15) identified ζ-carotene desaturase (ZDS), lycopene β-cyclase (LYC-β or CYC-β) and two β-carotene β-hydroxylases (CrtR-β). These enzymes sequentially function in the synthesis of carotene and its xanthophylls derivatives (60), notably lycopene and β-carotene, the two carotenoids that significantly accumulate in tomato plants overexpressing a pepper plastoglobulin (61). Interestingly, it has been described that ZDS requires plastoquinone as a cofactor (67,68), some of which is also located in plastoglobules (see above). Thus, these results suggest that plastoglobules are not only involved in carotenoid storage but actively participate in carotenoid metabolism in chromoplasts.

CONCLUDING REMARKS

In the last few years plastoglobules have started to emerge from obscurity and to become a charted and exciting new site on the chloroplast map (69). Apparently, plastoglobules are not simply lipid storage droplets but complex assemblies with roles in prenylquinone and carotenoid metabolism now outlined. Many of the proteins in the plastoglobule proteome, often predicted to be enzymes somehow involved in lipid metabolism, still remain functionally unassigned. While some of the plastoglobule lipid components are now known (Table 1), more certainly remain to be discovered. It will be exciting to explore the functional links between the plastoglobule lipids and the yet unassigned plastoglobule enzymes

and to define their roles in thylakoid formation and disassembly. What biochemical tricks will the plastoglobules still pull out of the hat for us?

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