Plastoglobuli: Plastid Microcompartments with Integrated Functions in Metabolism, Plastid Developmental Transitions, and Environmental Adaptation

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Abstract

Plastoglobuli (PGs) are plastid lipoprotein particles surrounded by a membrane lipid monolayer. PGs contain small specialized proteomes and metabolomes. They are present in different plastid types (e.g., chloroplasts, chromoplasts, and elaioplasts) and are dynamic in size and shape in response to abiotic stress or developmental transitions. PGs in chromoplasts are highly enriched in carotenoid esters and enzymes involved in carotenoid metabolism. PGs in chloroplasts are associated with thylakoids and contain ~30 core proteins (including six ABC1 kinases) as well as additional proteins recruited under specific conditions. Systems analysis has suggested that chloroplast PGs function in metabolism of prenyl lipids (e.g., tocopherols, plastoquinone, and phylloquinone); redox and photosynthetic regulation; plastid biogenesis; and senescence, including recycling of phytol, remobilization of thylakoid lipids, and metabolism of jasmonate. These functionalities contribute to chloroplast PGs' role in responses to stresses such as high light and nitrogen starvation. PGs are thus lipid microcompartments with multiple functions integrated into plastid metabolism, developmental transitions, and environmental adaptation. This review provides an in-depth overview of PG experimental observations, summarizes the present understanding of PG features and functions, and provides a conceptual framework for PG research and the realization of opportunities for crop improvement.

Contents

1. WHAT ARE PLASTOGLOBULI?

Plastoglobuli (PGs): plastid lipoprotein particles surrounded by a membrane lipid monolayer with protein and filled with hydrophobic molecules

Plastoglobuli (PGs) are lipoprotein particles surrounded by a membrane lipid monolayer and present in the plastids of most nonphotosynthetic and photosynthetic plant tissues, as well as those in moss and algae. PGs are lipid rich and contain sets of specialized hydrophobic metabolites and specific proteins, many of which have known or predicted enzymatic functions. In higher-plant chloroplasts, PGs are contiguous with the outer lipid leaflet of the thylakoid membrane, facilitating exchange of hydrophobic metabolites between the thylakoid membrane and the PGs.

Chloroplast PGs are typically ~30–500 nm in diameter. Their size can reversibly increase several-fold during various abiotic stresses and developmental transitions, such as senescence. PGs also often accumulate in plastid loss-of-function mutants, particularly those with defects in thylakoid formation. The dynamic behavior of PGs suggests that they play functional roles in chloroplast biogenesis, stress responses, and thylakoid breakdown. PGs in chromoplasts of red fruit (e.g., tomato and peppers) or red or orange petals and stamens (e.g., in *Crocus sativus*)

are of either tubular or fibrillar structure. Chromoplast PGs sequester the majority of chromoplast carotenoids and are enriched in carotenoid metabolic enzymes. PGs in nonphotosynthetic lipid-rich leucoplasts (in, e.g., trichomes and white petals) likely have specialized but unknown functions. PGs in elaioplasts of tapetum cells are enriched in sterol esters required for pollen coat assembly. However, the PG proteomes of these colorless, nonphotosynthetic plastids have not been determined.

PGs in chloroplasts were initially recognized in the 1950s and 1960s by transmission electron microscopy (TEM) of thin sections. Taking advantage of PGs' high lipid-to-protein ratio, investigators purified yellow PGs using flotation density centrifugation and then determined the main PG lipid classes (8, 44, 80, 105). The lipids of chloroplast PGs consist mainly of prenylquinones [in particular, plastoquinone-9 (PQ-9)] and triacylglycerol (TAG) and are devoid of chlorophyll and β-carotene (8), as discussed below. By contrast, PGs from nonphotosynthetic plastids in orange or red flower petals are enriched in xanthophylls (oxygenated carotenoids) and carotenoid esters (81). The increased sensitivity of mass spectrometry–based metabolomics and proteomics techniques has allowed detailed characterization of PG protein and metabolite composition and content. Reverse genetics approaches, particularly in *Arabidopsis thaliana*, are allowing functional aspects of these ubiquitous lipid-rich plastid particles to be elucidated. Collectively, the results show that PGs serve as a lipid microcompartment for the synthesis, storage, and redistribution of subsets of isoprenoids and neutral lipids in plastids and play a role in the homeostasis of the plastid redox state. As described in subsequent sections, less well-understood functional connections exist among PGs, carbon metabolism, and plastid biogenesis.

Plastids: a family of tissue-specific organelles derived from the undifferentiated proplastid; the chloroplast is a specific plastid type present in photosynthetic tissues

Thylakoid membrane: an extensive membrane system that harbors the photosynthetic machinery inside the chloroplast

Prenylquinones: a family of small molecules that contain a redox-active quinone head group and isoprenoid tail

Metabolomics: the study of metabolites at the systems level

Proteomics: the study of proteins at the systems level

2. VISUALIZATION OF PLASTOGLOBULI IN DIFFERENT TYPES OF PLASTIDS

PGs have different forms and functions depending on plastid type and function (82). PGs in chloroplasts (**Figure 1*c***) have been observed in the leaves of a wide variety of plant species and in various green and blue-green algae [e.g., *Pleurococcus*, *Nostoc*, *Chlamydomonas reinhardtii* (**Figure 1*j***), and *Dunaliella bardawil*] (62, 80). **Figure 1** shows images of PGs from different species, plastid types, mutants, and abiotic conditions. Chloroplast PGs are typically round (globular) with diameters of 50–200 nm, although the sizes can depend on the development stage, organ, and/or plastid type. Under abiotic stress or senescence conditions, and in the leaves of *Ficus* evergreen perennial plants, chloroplast PGs can reach sizes of up to 4 μm.

Generally, few systematic differences in chloroplast PG shape or size have been observed across species. However, chloroplast PGs in late-stage senescing tissue (cotyledons, leaves, or flower tissues) show wrinkled surfaces and differences in osmium tetroxide (OsO_4) staining intensity in TEM images (referred to as osmiophilicity), likely reflecting changes in prenyl lipid and neutral lipid content (91, 144). Several studies have observed that purified PGs do not easily coalesce, as can be seen in scanning electron microscopy (SEM) images of purified PGs (90) (**Figure 1*b***). Chloroplast PGs are ubiquitous in algae (**Figure 1*j***), moss, and angiosperms, underscoring the evolutionary conservation of these structures (see Section 5).

Chromoplasts are yellow, orange, or red pigmented plastids in colored fruits, flowers (petals or stamen), or specialized storage roots. PGs in chromoplasts have been most extensively studied in red bell pepper (*Capsicum annuum*) (26, 46, 110, 116, 162) and in colored petals from various plant species (1, 36, 48, 81, 145) (**Figure 1*g*,*b***). Ultrastructural studies of chromoplasts from a wide range of species and various organs revealed different internal structures, assigned as globules, crystals, membranes, fibrils, and tubules (17, 155). For instance, chromoplasts of red pepper accumulate fibrillar PGs (**Figure 1*g***), whereas tomato fruit PGs are globular (126). Variations in the relative

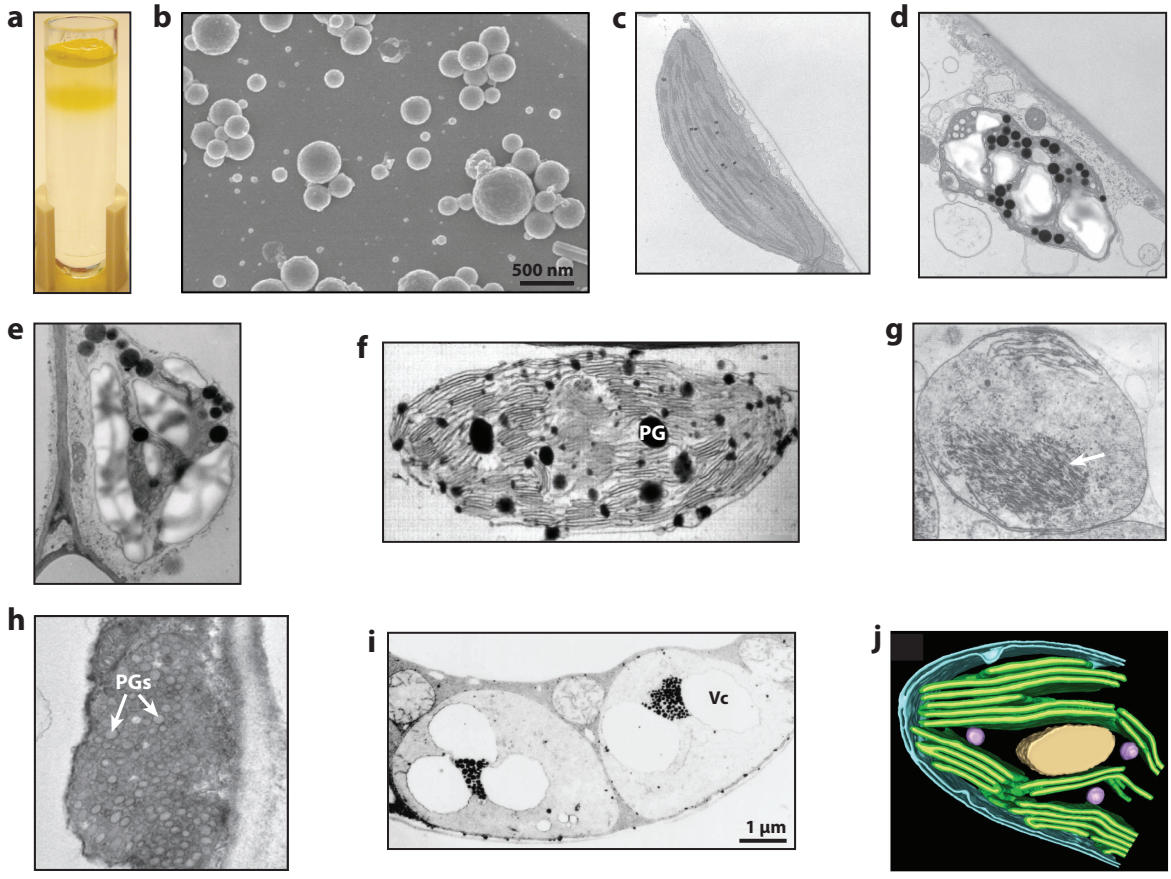


Figure 1

PGs in different species, plastid types, mutants, and abiotic conditions. (*a*) PGs isolated from *Arabidopsis* leaf chloroplasts by sucrose gradient centrifugation; note the yellowish color. Adapted from Reference 162. (*b*) Scanning electron microscopy image of purified PGs from *Arabidopsis* leaf chloroplasts. Adapted from Reference 90. (*c*) *Arabidopsis* leaf chloroplast. (*d*) *Arabidopsis* leaf chloroplast after 3 days of high-light stress. (*e*) *Arabidopsis* leaf chloroplast under nitrogen deprivation. Adapted from Reference 39. (*f*) Senescing *Arabidopsis* leaf chloroplast with large PGs. Adapted from Reference 63. (*g*) Red pepper fruit chromoplasts containing fibrillar PGs (*white arrow*). Adapted from Reference 25. (*h*) Tomato petal chromoplast filled with globular PGs. Adapted from Reference 2. (*i*) PG clusters from the chloroplast biogenesis mutant *tatc*. Adapted from Reference 102. (*j*) Three-dimensional segmentation of an electron tomographic volume of a *Chlamydomonas* chloroplast, showing PGs in purple. Adapted from Reference 32. Abbreviations: PG, plastoglobule; *tatc*, *twin-arginine translocation c*; Vc, vacuole-like structure.

Fibrillin (FBN): a hallmark protein family of plastoglobuli (although several members are not located in plastoglobuli); fibrillins are conserved from cyanobacteria

content of carotenoids and their esterification, polar lipids, and proteins likely drive these structural differences (e.g., 17, 84, 126). An example of how a red pepper protein can induce fibrillar PGs in tomato chromoplasts, rather than the natural globular PGs, demonstrated the importance of proteins in the formation of PGs (126).

Elaioplasts in tapetal cells within anthers are small, round plastids with membrane-associated PGs that serve as a source of sterol esters for the pollen coat. PG elaioplasts have been visualized in particular in tapetum cells from *Brassica rapa* (154), *Brassica napus* (50, 156), and *Brassica campestris* (141) (**Figure 1**). Live imaging of green fluorescent protein (GFP) fused to the PG protein FIBRILLIN 1a (FBN1a) tracked the development and disappearance of PGs in elaioplasts of tapetum cells of *B. napus* (138). Elaioplasts are also present in the cotyledons of oilseeds, where

their stored oils are converted into carbohydrates through gluconeogenesis during germination and early seedling development. The proteomes of elaioplast PGs from either seeds or tapetal cells have not been determined but would help to better identify their metabolic functions and pathways.

Nonphotosynthetic plastids in roots are small, with minor, poorly defined internal structures, including PGs. Root PG size and number visibly increased in an *Arabidopsis* plastid protease mutant (167), suggesting that PGs also play a role in homeostasis in root plastids. Lipid-rich structures in the starch-filled amyloplasts in storage organs are small and have not been studied.

De-etiolation: a transition that dark-grown plants undergo in response to light; it converts etioplasts to chloroplasts

3. DYNAMICS OF PLASTOGLOBULI DURING DEVELOPMENTAL TRANSITIONS AND STRESS

One of the striking features of PGs is their incredibly dynamic nature: They can rapidly increase or decrease in size during developmental transitions or in response to changes in environmental conditions. In this section, we discuss key examples of such dynamics.

3.1. De-etiolation

Etioplasts contain globular PGs, usually in proximity to or embedded within the paracrystalline prolamellar bodies that contain protochlorophyllide reductase and lipids (137). During de-etiolation, the abundances of PGs and prolamellar bodies decrease concomitantly with thylakoid formation; this suggests that PGs contribute to thylakoid development, most likely by providing carotenoids, prenylquinones, and possibly TAG for membrane lipid synthesis. Note that the compositions of PGs and prolamellar bodies are not identical, because tandem mass spectrometry analysis of low-density structures purified from rice etiolated seedlings (162) or dark-grown wheat (*Tritium* spp.) leaves (12) showed that these structures have proteomes that differ substantially from those of chloroplast PGs (162).

3.2. Senescence

During leaf senescence, thylakoid membranes, their protein complexes, and associated cofactors are dismantled in a controlled fashion (6, 10, 51, 53, 57, 74). TEM and SEM have been used to visualize this process, during which PGs for various species showed a dramatic supersizing (40, 45, 80, 140, 144) (**Figure 1*f***). One of the first and most detailed characterizations of the PG metabolite composition at different stages of natural senescence in leaves was in beech (*Fagus sylvatica*) natural tree stands (140) (for details, see Section 4). There is a strong correlation between thylakoid dismantling and supersizing of PGs. In particular, prenylquinones and free fatty acids, but not glycolipids or proteins, accumulate in PGs during the senescence process (140 and references therein). However, the carotenoid and carotenoid ester content of PGs, which is very low in green leaves, increases in early stages of senescence (while total leaf carotenoid content decreases) and then decreases to low levels in more advanced stages of senescence (140). More recent work has also shown that PGs play a role in chlorophyll degradation and phytol recycling (151) (see Section 7). Several reports have suggested that PGs also serve as deposits for protein degradation fragments of the photosynthetic machinery (40, 132, 133). However, as detailed by Ytterberg et al. (162), it is more likely that low-density thylakoid fragments contaminate the PGs.

Various reports have suggested that PGs are released from the senescing chloroplast, either following rupture of the chloroplast envelope or through some other mechanism (82, 85, 147). These released PGs would then be digested in, e.g., the vacuoles (82). A recent report with

high-resolution TEM images of senescing tepal epidermal cells in *Iris* flowers and senescing watermelon leaves made a convincing case for the departure of lipid-rich bodies from gerontoplasts to the cytosol (85, 147). PG-containing vesicles formed at the chloroplast envelope and were then expelled from chloroplasts into the cytoplasm. Lipid droplets in the vacuole shared a similar ultramicroscopic appearance with PGs in chloroplasts, suggesting that PGs were engulfed and degraded by the vacuole after they were secreted from chloroplasts (85). It is not clear how these observations relate to various types of autophagy-independent vesicle degradation systems or to chloroplast macroautophagy (chlorophagy) (57, 58, 153, 157).

3.3. The Chloroplast-to-Chromoplast Transition

Chromoplasts develop from chloroplasts and therefore undergo a degreening process, during which thylakoid membranes and their contents are removed and/or recycled (29). During this transition, PGs accumulate carotenoids and other prenyl lipids through de novo synthesis as well as remobilization from the thylakoid and envelope membranes (26, 126). This is a well-controlled developmental plastid transition and has been studied extensively in ripening carotenoid-bearing fruit (e.g., peppers, tomato, and sweet orange) through genetics, in particular in tomato (60, 86, 104) and to a lesser degree in colored petals of, e.g., cucumber (*Cucumis sativus*) (131, 145).

3.4. Abiotic Stress

When plants are exposed to drought or high-light stress (**Figure 1*d***) or transferred to nitrogen-limiting conditions (**Figure 1*e***), PG size rapidly increases; upon stress relief, it decreases again (34, 39, 165). These changes in PG size and, frequently, the extent of OsO_4 staining in TEM likely reflect metabolite exchange between the thylakoid membrane and PGs and, possibly, de novo metabolite synthesis and export. Changes in major PG metabolites, such as tocopherols (vitamin E), various quinones, phytol esters, and TAGs (see Section 4), contribute to the reversible change in PG size and properties.

Finally, many thylakoid or chloroplast biogenesis mutants show increased accumulation of PGs, e.g., in intrachloroplast protein sorting mutants [such as the *twin-arginine translocation c* (*tatc*) mutant; **Figure 1*i***] and chloroplast protease mutants (7, 70, 119). These phenotypes are typically accompanied by a reduced amount of thylakoid membranes. Whether the metabolite and/or protein content of PGs is the same in these mutants as it is in wild-type plants is unknown.

4. MOLECULAR PROFILING OF PLASTOGLOBULI IN PLANTS AND ALGAE

4.1. Overview of Metabolite Content in Plastoglobuli

The small-molecule content of various types of PGs was characterized initially using combinations of thin-layer chromatography, high-pressure liquid chromatography, and absorption/emission spectroscopy (8, 44, 48, 80, 136, 140, 156) and subsequently by gas chromatography (GC) and liquid chromatography (LC) mass spectrometry (2, 24, 39, 50, 83, 91, 95–97, 118, 126, 136, 140, 148, 164) and nuclear magnetic resonance (2). **Table 1** summarizes information about the metabolite content of PGs from various sources and references; the relative distributions of these small molecules depend greatly on the plastid type and developmental state.

Three main classes of small molecules are present in PGs: neutral lipids (in particular, TAG, phytol esters, and free fatty acids), prenylquinones [in particular, α-tocopherol, PQ-9,

Table 1 Small-molecule content in isolated PGs from different organs and developmental states in plants and algae

Metabolite	Chloroplast (in leaves)	Chromoplast (in red fruit and flower organs)
Neutral lipids		
Galactolipids (MDDG and DGDG)	Trace levels[a]	Low levels
Sulfoquinovosyl diacylglycerol	Trace levels	Low levels
Phospholipids	Trace levels	Low levels
Triacylgycerol	High levels; decreasing levels with progressive senescence[b]	Extremely high levels
Sterol esters	—[c]	—
Free fatty acids	Moderate levels; increasing levels during senescence	—
Tocopherols and quinones		
α-Tocopherol	Moderate levels	Moderate levels
α-Tocopherolquinol	Moderate levels	Low levels
Plastochromanol-8	Moderate levels	Low levels
Plastoquinol-9	Major component	Significant levels
Phylloquinone (vitamin K_1)	Moderate levels	Low levels
Carotenoids and derivatives		
Linear carotenoids (lycopene)	Trace levels	High levels of lycopene
Cyclic carotenoids (lutein and the xanthophylls zeaxanthin, antheraxanthin, violaxanthin, and neoxanthin)	Trace levels	High levels of lycopene
Keto-xanthophylls (capsanthin and capsorubin)	—	Only in pepper species, which have extremely high levels of capsanthin and significant levels of capsorubin
Carotenoid esters	Trace levels	75–90% of carotenoids
Chlorophyll catabolite		
Fatty acid phytol ester	Trace levels; high levels from chlorophyll breakdown during senescence	—

Dashes indicate that the metabolite is not present in that organ. Abbreviations: DGDG, digalactosyldiacylglycerol; MGDG, monogalactosyldiacylglycerol; PG, plastoglobule.
[a]Low levels detected in elaioplast PGs in tapetum cells.
[b]Moderate levels detected in elaioplast PGs in tapetum cells.
[c]High levels detected in elaioplast PGs in tapetum cells.

plastochromanol-8 (PC-8), and phylloquinone (phylloQ, also known as vitamin K_1)] and various carotenoids and apocarotenoids. (**Table 1**). Although earlier studies detected galactolipids in isolated leaf PGs (8, 44, 80), these galactolipids may in large part originate from contaminating thylakoid membranes (as discussed in 136). It can be assumed that apolar components are buried in the interiors of PGs and are covered by polar lipids and proteins at the surfaces of PGs (48). Changes in PG metabolite content affect PG size and shape and are also reflected in the osmiophilicity in TEM images (91), as was nicely observed for PGs isolated from apple leaves of an *FBN4* RNA interference (RNAi) line (128): The PGs of the *fbn4* mutant had <10% of wild-type PQ-9, although the total leaf PQ-9 content was unchanged. Because the solanesyl side chain of

PQ-9 is highly unsaturated and reacts with OsO_4, loss of PQ-9 results in lower osmiophilicity and less contrast in TEM.

The first in-depth and comparative quantitative metabolite analyses were of highly purified chloroplast PGs from natural senescing beech leaves and nonphotosynthetic plastids from yellow petals of common broom (*Sarothamnus scoparius* L., also named *Cytisus scoparius*) (136, 140). Oxidized and reduced PQ-9 and TAG were the most dominant metabolites in chloroplast PGs, followed by free fatty acids, α-tocopherol, tocoquinone, and phylloQ; only very small amounts of chlorophylls and carotenoids or lipids (galactolipids or phospholipids) were observed. During four stages of natural senescence of beech leaves, the TAG content dramatically declined in PGs, whereas carotenoids (mostly in esterified form) and free fatty acid levels strongly increased. PQ-9 was oxidized during senescence (140). By comparison, PGs from nonphotosynthetic plastids present in yellow petals of broom were completely dominated by TAG (60%) and carotenoid esters (32%), with low levels of PQ-9 (~2.5%), α-tocopherol (~0.7%), and phylloQ and tocoquinone (~0.1–0.2%) (136). Free fatty acids were not detected.

During chromoplast differentiation in red tomato and pepper fruits, massive biosynthesis of carotenoids gives rise to the red fruit color. The carotenoids accumulate in the hydrophobic core of globular, tubular, or fibrillar PGs in chromoplasts. Quantitative analysis of red pepper fruit (26) showed that the most abundant carotenoid in red pepper fibrils is capsanthin (55%) followed by similar levels (4–6%) of violaxanthin, β-carotene, capsorubin, β-cryptoxanthin, and zeaxanthin as well as minor amounts of other carotenoids. The degree of esterification with fatty acids of the carotenoids varied from 75% to 100% and was 95% in the case of capsanthin, and is thought to have a stabilizing effect on xanthophylls (2). Moreover, the increased hydrophobicity conferred by the acylation may favor sequestration in the hydrophobic cores of PGs and carotenoid fibrils. Other than carotenoids (1,000 nmol/mg protein), the red pepper chromoplast fibrils contained phospholipids (70 nmol/mg protein), monogalactosyldiacylglycerol (900 nmol/mg protein), digalactosyldiacylglycerol (460 nmol/mg protein), and tocopherols (70 nmol/mg protein) (26).

Lipid bodies in elaioplasts of tapetal cells accumulate hydrophobic substances, including sterol esters, a major component of the pollen coat. The sterol head groups and the acyl chains of sterol esters in the pollen coat and plastid lipid bodies are very similar in composition, suggesting that the lipid bodies are the source of the sterol esters in the pollen coat (50, 156).

In subsequent sections, we discuss the proteins and enzymes involved in the generation of these various small molecules. We also provide overviews of their relevant metabolic pathways and functionalities.

4.2. Overview of Protein Content in Plastoglobuli in Vascular Plants

PGs are enriched in a set of ~30 proteins, as demonstrated by in-gel staining, immunoblotting, and mass spectrometry of purified PGs (**Table 2**). The first reports of PG-enriched proteins were from red bell pepper (*C. annuum*) chromoplasts (46) and a 35-kDa carotenoid-associated protein from cucumber (*Cucumis sativus*) corollas (130, 145); however, their molecular identities could not be determined. The first identified PG-enriched protein was from red pepper fruit chromoplasts and was a member of the FBN family, a name based on its association with the fibrillar carotenoid-enriched structures in these chromoplasts (25, 26). FBN homologs were subsequently identified in other plants and given various names, including PLASTOGLOBULIN (PGL) (15), PLASTID-LIPID ASSOCIATED PROTEIN (PAP) (75), FIBRILLIN (FIB) (161), and CHR (150) proteins, but FBN has become the de facto preferred nomenclature and abbreviation (e.g., 37, 38, 66, 117). We use that abbreviation in this review and strongly suggest that others also adopt it, as mentioned by Singh & McNellis (129) (**Table 2**).

Table 2 Proteins highly enriched in PGs from *Arabidopsis* chloroplasts and identification of their orthologs in PGs isolated from the green alga *Dunaliella bardawil*, red bell pepper chromoplasts, and *Chlamydomonas reinhardtii* eyespots, along with non-PG-localized proteins of the ABC1K and FBN families

Gene identifier	Protein name (previous names or orthologs)	Location in *Arabidopsis*[a]	Relative abundance in chloroplast PGs[b]	Known or postulated function[c]	Phosphorylated[d]	GreenCut2[e]	*D. bardawil* PGs[f]	Red bell pepper chromoplast PGs[g]	*C. reinhardtii* eyespot PGs[h]
Proteins highly enriched in PGs									
AT4G31390	ABC1K1 (PGR6)	Chloroplast core PGs	6	Kinase			Yes		Yes
AT1G79600	ABC1K3 (ACDO1)	Chloroplast core PGs	7	Kinase			Yes		Yes
AT1G71810	ABC1K5	Chloroplast core PGs	18	Kinase					
AT3G24190	ABC1K6 (EYE3)	Chloroplast core PGs	24	Kinase		Yes	Yes		
AT3G07700	ABC1K7 (SIA1)	Chloroplast core PGs	27	Kinase in senescence			Yes		
AT5G05200	ABC1K9	Chloroplast core PGs	5	Kinase		Yes	Yes	Yes	
AT4G19170	CCD4	Chloroplast core PGs	9	Carotenoid cleavage, senescence					
AT1G54570	PES1 (DGAT3, PYP1)	Chloroplast core PGs	11	Acetyltransferase/esterase, senescence				Yes	
AT3G26840	PES2 (DGAT4)	Chloroplast core PGs	23	Acetyltransferase/esterase, senescence					
AT5G41120	Esterase 1 (ELT4)	Chloroplast core PGs	30	Unknown					
AT4G04020	FBN1a (PGL35)	Chloroplast core PGs	1	Structural, stress, small-molecule transport	Yes			Yes	Yes
AT4G22240	FBN1b (PGL33)	Chloroplast core PGs	3	Structural, stress, small-molecule transport	Yes			Yes	Yes
AT2G35490	FBN2 (PGL40)	Chloroplast core PGs	4	Structural, stress, small-molecule transport	Yes			Yes	Yes

(*Continued*)

Table 2 (*Continued*)

Gene identifier	Protein name (previous names or orthologs)	Location in *Arabidopsis*[a]	Relative abundance in chloroplast PGs[b]	Known or postulated function[c]	Phosphorylated[d]	GreenCut2[e]	*D. bardawil* PGs[f]	Red bell pepper chromoplast PGs[g]	*C. reinhardtii* eyespot PGs[h]
AT3G23400	FBN4 (PGL30.4)	Chloroplast core PGs	2	Structural, stress, small-molecule transport	Yes		Yes	Yes	Yes
AT3G58010	FBN7a	Chloroplast core PGs	8	Structural, stress, small-molecule transport					Yes
AT2G42130	FBN7b	Chloroplast core PGs	13	Structural, stress, small-molecule transport	Yes				Yes
AT2G46910	FBN8	Chloroplast core PGs	17	Structural, stress, small-molecule transport		Yes	Yes		Yes
AT1G32220	Flavin reductase related 1	Chloroplast core PGs	14	Unknown	Yes		Yes	Yes	
AT2G34460	Flavin reductase related 2	Chloroplast core PGs	21	Unknown	Yes	Yes	Yes		
AT3G27110	M48 protease	Chloroplast core PGs	29	Peptidase, senescence			Yes		Yes
AT1G06690	NAD(P)-aldo/keto-reductase	Chloroplast core PGs	20	Unknown	Yes	Yes	Yes	Yes	
AT5G08740	NDC1	Chloroplast core PGs	12	Quinone reductase, phylloquinone biosynthesis			Yes		Yes
AT3G10130	SOUL domain–containing HBP3	Chloroplast core PGs	16	Unknown			Yes		Yes
AT1G78140	UbiE methyltransferase–related 1	Chloroplast core PGs	19	Potential methyltransferase (as VTE3)					Yes
AT2G41040	UbiE methyltransferase–related 2	Chloroplast core PGs	22	Potential methyltransferase (as VTE3)		Yes	Yes		Yes
AT4G13200	Unknown 1	Chloroplast core PGs	15	Unknown	Yes			Yes	

AT3G43540	Unknown 2 (DUF1350)	Chloroplast core PGs	26	Unknown			Yes		
AT1G73750	Unknown SAG	Chloroplast core PGs	28	Unknown senescence-associated function					
AT4G32770	VTE1	Chloroplast core PGs	10	Tocopherol metabolism	In cTP?	Yes	Yes	Yes	
AT1G28150	Unknown	Chloroplast PGs[i]		Unknown					Yes
AT1G52590	Unknown	Chloroplast PGs[j]		Oxidoreductase			Yes		
AT3G25760	AOC1	Recruited to chloroplast PGs		Jasmonate biosynthesis					
AT3G25770	AOC2	Recruited to chloroplast PGs		Jasmonate biosynthesis					
AT5G42650	AOS	Recruited to chloroplast PGs		Jasmonate biosynthesis	Yes				
AT3G45140	LOX2	Recruited to chloroplast PGs		Jasmonate biosynthesis	Perhaps				
AT1G17420	LOX3	Recruited to chloroplast PGs		Jasmonate biosynthesis					

(*Continued*)

Table 2 (*Continued*)

Gene identifier	Protein name (previous names or orthologs)	Location in *Arabidopsis*[a]	Relative abundance in chloroplast PGs[b]	Known or postulated function[c]	Phosphorylated[d]	GreenCut2[e]	*D. bardawil* PGs[f]	Red bell pepper chromoplast PGs[g]	*C. reinhardtii* eyespot PGs[h]
AT1G72520	LOX4	Recruited to chloroplast PGs		Jasmonate biosynthesis					
AT5G13800	PPH	Senescing chloroplast PGs		Chlorophyll degradation, senescence					
AT3G04870	ZDS	Chromoplast PGs		Carotenoid biosynthesis				Yes	
AT3G10230	LYC-β	Chromoplast PGs		Carotenoid biosynthesis			Yes	Yes	
AT4G25700/ AT5G52570	CrtR-β	Chromoplast PGs (but has a TMD)[k]		Carotenoid biosynthesis				Yes	
Non-PG-localized proteins of the ABC1K and FBN families									
AT5G24970	ABC1K2	Plastid		Kinase					
AT2G39190	ABC1K4	Plastid		Kinase					
AT5G64940	ABC1K8 (OSA1, ABC1–2)	Envelope		Kinase		Yes			
AT1G51110	FBN10	Thylakoid, etioplast PGs/PLBs[l]		Structural, stress, small-molecule transport		Yes			
AT5G53450	FBN11/ORG1[m]	Plastid		Structural, stress, small-molecule transport, kinase					
AT3G26070	FBN3a	Thylakoid		Structural, stress, small-molecule transport	Yes	Yes			
AT3G26080	FBN3b	Thylakoid		Structural, stress, small-molecule transport		Yes			
AT5G09820	FBN5	Stroma		PQ-9 synthesis (solanesyl moiety)					

AT5G19940	FBN6	Thylakoid		Structural, stress, small-molecule transport		Yes			
AT4G00030	FBN9	Plastid		Structural, stress, small-molecule transport	Perhaps	Yes			
AT1G18060	FBN-like	Thylakoid		Unknown					
AT1G11390	ABC1K10-a	Mitochondria		Kinase					
AT1G61640	ABC1K10-b	Mitochondria		Kinase					
AT5G24810	ABC1K11 (TaABC1)	Mitochondria		Kinase					
AT4G24810	ABC1K12-a	Mitochondria		Kinase		Yes			
AT5G50330	ABC1K12-b	Mitochondria		Kinase		Yes			
AT4G01660	ABC1K13	Mitochondria		Kinase, ubiquinone synthesis					
AT1G65950	ABC1K14	Mitochondria		Kinase					
AT2G40090	ABC1K15	Mitochondria		Kinase					

Abbreviations: ABC, ABERRANT CHLOROPLAST DEVELOPMENT; ABC1K, ACTIVITY OF BC1 COMPLEX KINASE; ACDO1, ABC1-LIKE KINASE RELATED TO CHLOROPHYLL DEGRADATION AND OXIDATIVE STRESS 1; AOC, ALLENE OXIDE CYCLASE; AOS, ALLENE OXIDE SYNTHASE; CCD4, CAROTENOID CLEAVAGE DIOXYGENASE 4; CrtR-β, β-CAROTENE β-HYDROXYLASE; DGAT, ACYL-COA:DIACYLGLYCEROL ACYLTRANSFERASE; DUF1350, DOMAIN OF UNKNOWN FUNCTION 1350; ELT4, ESTERASE/LIPASE/THIOESTERASE 4; FBN, FIBRILLIN; HBP3, HEME BINDING PROTEIN 3; LOX, LIPOXYGENASE; LYC-β, lycopene β-cyclase; NDC1, NADP(H) DEHYDROGENASE C1; ORG1, OBP3-RESPONSIVE GENE 1; OSA1, OXIDATIVE STRESS-RELATED ABC1-LIKE PROTEIN 1; PES, PHYTOL ESTER SYNTHASE; PG, plastoglobule; PGL, PLASTOGLOBULIN; PGR6, PROTON GRADIENT REGULATION 6; PLB, prolamellar body; PPH, PHEOPHYTIN PHEOPHORBIDE HYDROLASE; PQ-9, PLASTOQUINONE-9; PYP1, PALE YELLOW PETAL 1; SAG, SENESCENCE-ASSOCIATED GENE; SIA1, SALT-INDUCED ABC1 KINASE 1; TMD, transmembrane domain; VTE1, TOCOPHEROL CYCLASE (VITAMIN E DEFICIENT 1); ZDS, ζ-CAROTENE DESATURASE.

[a]Indicates the curated location based on all available experimental information (see also the Plant Proteome Database at **http://ppdb.tc.cornell.edu**). Assignment of proteins as PG core proteins is from Lundquist et al. (89).

[b]Indicates the relative abundance of PG proteins based on label-free spectral counting (89); a blank cell indicates that no data on relative abundance are available.

[c]Indicates the experimentally determined or postulated functions for each protein.

[d]Indicates whether experimental support has been found for phosphorylation in *Arabidopsis* (88); a blank cell indicates that no evidence has been found for that protein.

[e]Indicates whether the protein was reported as part of GreenCut2 (61); a blank cell indicates that it was not reported.

[f]Indicates whether the protein was reported in the PG proteome of *D. bardawil* (23); a blank cell indicates that it was not reported.

[g]Indicates whether the protein was reported in the PG proteome of red bell pepper (162); a blank cell indicates that it was not reported.

[h]Indicates whether the protein was reported in the eyespot proteome of *C. reinhardtii* (69, 124, 152); a blank cell indicates that it was not reported.

[i]This protein was originally reported by Ytterberg et al. (162) and was recently confirmed as a PG protein (K.J. van Wijk, unpublished data).

[j]This protein was originally reported by Vidi et al. (148) and was recently confirmed as a PG protein (K.J. van Wijk, unpublished data).

[k]This protein is predicted to have a TMD and therefore cannot be part of monolayer particles. We expect that it is located in membranes copurified with chromoplast PGs.

[l]This protein was identified in a low-density fraction enriched for PLBs from rice etioplasts (162).

[m]This protein has a high-confidence protein kinase domain and a much higher molecular mass (76 kDa) than the rest of the FBN family (25–45 kDa).

ACTIVITY OF BC1 COMPLEX KINASE (ABC1K): a family of atypical kinases in bacteria, mitochondria, and chloroplasts that regulate prenylquinone metabolism and other pathways

Tocopherol cyclase: a key enzyme in tocopherol and plastochromanol-8 biosynthesis as well as tocopherol recycling

Phytol ester synthase (PES): a senescence-induced enzyme that recycles chlorophyll and lipid degradation products (phytol and free fatty acids, respectively)

A breakthrough in the systematic identification of the PG proteome came with improvements and commercialization of mass spectrometry. These developments allowed the identification of proteins enriched in purified PGs from chloroplasts of *Arabidopsis* (90, 91, 148, 162), maize (*Zea mays*) (56), and *D. bardawil* (23); from naturally senescing leaves of *Arabidopsis* (11); and from the chromoplasts of red bell pepper (162). **Table 2** provides an overview (with key references) of the highly enriched and specialized proteome of PGs from selected species. Chloroplast PGs contain a highly specialized proteome of ~30 proteins; most of these proteins are located almost exclusively in PGs, and an additional small set of proteins (involved in chlorophyll degradation and jasmonate metabolism) are recruited under specific conditions (91). None of the PG proteins have known or predicted transmembrane domains, which is consistent with the PGs being bounded by a membrane lipid monolayer (protein transmembrane domains require a lipid bilayer for insertion). Therefore, PG core proteins localize at the periphery of PGs, probably by inserting short hydrophobic domains into the hydrophobic core or through interactions with other PG proteins, depending on their function (4, 65, 110).

Using publicly available *Arabidopsis* genome-wide mRNA data, Lundquist et al. (90) generated a coexpression network using the PG core proteins as nodes. This network analysis suggested four major coexpression modules, each showing functional enrichment, including enrichment in senescence, chloroplast proteolysis, carotenoid metabolism, chloroplast redox regulation, the Calvin-Benson cycle, and chloroplast biogenesis. This analysis helped to associate the PG core proteins with different processes or functions; where relevant, we refer further to this coexpression network below. We also briefly comment on these PG proteins below; more detailed discussions are provided in later sections, including detailed information on relevant biosynthetic pathways.

The identified functions of PG proteins concern mainly the regulation of isoprenoid metabolism and remobilization of thylakoid lipids and fatty acids. **Figure 2** integrates present knowledge of six PG protein functions and PG metabolites in a schematic overview of metabolic pathways in different PG types; in later sections, **Figure 3–8** provide additional detail.

The most abundant proteins in chloroplast PGs are specific members of the plastid-specific FBN family (129) and members of the ACTIVITY OF BC1 COMPLEX KINASE (ABC1K) family (89), which represent ~53% and ~19% of the PG protein mass, respectively (90). FBNs share limited sequence homology with lipocalins, which are small proteins involved in the binding and transport of small hydrophobic compounds (reviewed in 129). *Arabidopsis* has 14 FBNs, 7 of which (FBN1a, -1b, -2, -4, -7a, -7b, and -8) are considered PG core proteins (**Table 2**). The functions of PG-localized FBN1, -2, and -4 have been studied through loss-of-function mutants and/or overexpression (128, 160, 161) (for more detail, see Section 8). The other FBNs are located primarily in thylakoid membranes (FBN3a, -3b, -6, and -9 and FBN-like) or the chloroplast stroma (FBN5) (66, 90). The *Arabidopsis* ABC1K family includes 17 members, 6 of which (ABC1K1, -3, -5, -6, -7, and -9) are located in PGs (**Table 2**). ABC1K2, -4, and -8 are localized elsewhere in the chloroplast, and ABC1K10–14 are located in the mitochondria (89). ABC1K homologs in bacteria and mitochondria phosphorylate components of the ubiquinone biosynthetic pathway, thereby regulating its activity (89). Studies of loss-of-function mutants have indicated that *Arabidopsis* PG-localized ABC1K1 and -3 affect prenyl lipid content in PGs and abiotic stress responses (91, 95, 96) (for more detail, see Section 9).

Other PG core proteins include the well-studied TOCOPHEROL CYCLASE [VITAMIN E DEFICIENT 1 (VTE1)] (108), which is a key enzyme in tocopherol and PC-8 biosynthesis, and PHYTOL ESTER SYNTHASE 1 (PES1) and PES2, which are involved in the formation of phytyl esters following cleavage of the phytol tail from chlorophyll and free fatty acids from galactolipids (83) (**Figure 2**). PGs also contain a third PES-like protein, AT5G41120

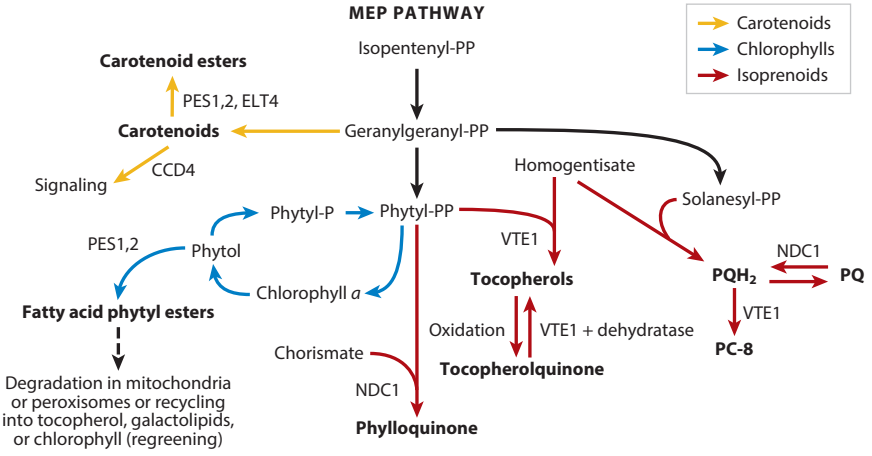


Figure 2

Integrated overview of metabolic pathways, six PG core enzymes, and how these enzymes function in isoprenoid metabolism and neutral lipid metabolism. Fatty acid donors of in vitro PES1 and -2 acyltransferase activity include 1,2- or 1,3-MGDG, acyl-CoA, and acyl-ACP. Abbreviations: ACP, acyl carrier protein; CCD4, CAROTENOID CLEAVAGE DIOXYGENASE 4; CoA, coenzyme A; ELT4, ESTERASE/LIPASE/THIOESTERASE 4; MEP, 2-*C*-methyl-D-erythritol 4-phosphate; MGDG, monogalactosyldiacylglycerol; NDC1, NADP(H) DEHYDROGENASE C1; P, phosphate; PC-8, plastochromanol-8; PES, PHYTOL ESTER SYNTHASE; PG, plastoglobule; PP, pyrophosphate; PQ, plastoquinone-9; PQH_2, plastoquinol-9; VTE1, TOCOPHEROL CYCLASE (VITAMIN E DEFICIENT 1).

[ESTERASE/LIPASE/THIOESTERASE 4 (ELT4)] (**Table 2**), but its function has not been studied. NADP(H) DEHYDROGENASE C1 (NDC1) reduces oxidized PQ-9 to PQ-9-H_2 within PGs (33) and plays an essential role as a reductase in phylloQ biosynthesis (35) (**Figure 2**). The low-abundance M48 protease is a member of the zinc metallopeptidase family and acts as a positive regulator of senescence, perhaps by degrading CCD4 (11). Other PG core proteins, such as methyltransferases, reductases, and the SOUL domain–containing HEME BINDING PROTEIN 3 (HBP3) (**Table 2**), have various predicted functional domains, but their functions have not been studied.

Proteome analysis of isolated PGs from the chromoplasts of ripe red peppers identified ξ-carotene desaturase (ZDS), lycopene β-cyclase (LYC-β), and two β-carotene β-hydroxylases (CrtR-βs) operating in series in bicyclic carotenoid biosynthesis, as well as nine homologs (including FBN1a, -1b, -2, and -4; PES1; ABC1K9; and VTE1) of proteins identified in chloroplast PGs (162) (**Table 2**). This suggests that PGs in chromoplasts have specific enzymatic functions in carotenoid biosynthesis (**Figure 2**), in addition to their well-known function of carotenoid storage and sequestration (25, 136, 150). The presence of VTE1 is in agreement with the low levels of tocopherol in chromoplast PGs (**Table 1**). Several chloroplast PG proteins were not found in chromoplast PGs [e.g., ABC1K1, -3, -5, and -7; PES2; ELT4; and CAROTENOID CLEAVAGE DIOXYGENASE 4 (CCD4)], which suggests that these proteins are involved in processes that are no longer required in the chromoplasts of mature red peppers (e.g., chlorophyll breakdown and electron transport). Finally, mass spectrometry analysis of low-density membranes of rice etioplasts revealed mostly protochlorophyllide reductase, which is abundant in prolamellar bodies, but also FBN10, which is located in PGs, embedded in prolamellar bodies, or associated with prethylakoid membranes (162).

5. THE ORIGIN AND EVOLUTION OF PLASTOGLOBULE STRUCTURES AND COMPONENTS

Electron-dense particles are visible in TEM not only in vascular plants, but also in nonvascular species, such as the moss *Physcomitrella patens* (115), green and blue algae (62, 124), and cyanobacteria. However, only in the case of the green alga *D. bardawil* have PGs been isolated and analyzed for metabolite and protein content (23, 24) (**Table 2**). Nevertheless, homologs of many of the higher-plant PG core proteins are present in lower photosynthetic organisms and are part of the set of "GreenCut" proteins found only in photosynthetic organisms (61, 100) (see **Table 2**). For instance, phylogenetic analysis of the ABC1K family showed that ABC1K proteins were already present in archaea but that the family greatly expanded in photosynthetic eukaryotes (89) (see Section 9). Most of the ABC1K proteins in plastids are derived from the cyanobacterial endosymbiont, whereas the PG core protein ABC1K9 likely derived from the recipient organism of the endosymbiont and belongs to a separate ancestral clade together with the mitochondrion-localized ABC1K10 (89) (**Figure 3**). Likewise, the phylogeny of the FBN family showed that the PG gene

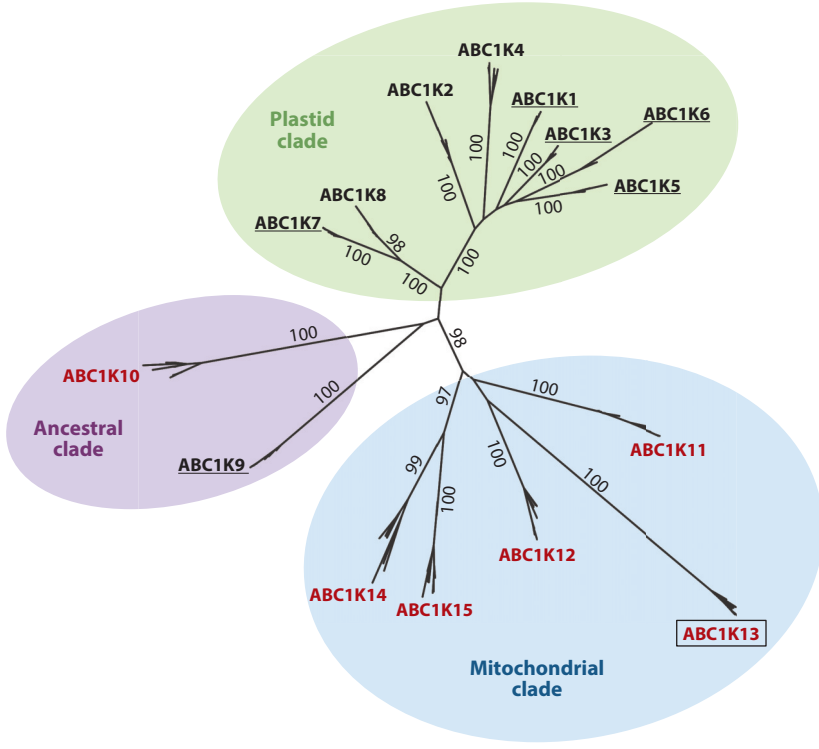


Figure 3

Phylogenetic unrooted tree of the angiosperm ABC1K proteins based on the amino acid sequence alignment of the 126 ABC1K proteins from three eudicot species (*Arabidopsis thaliana*, *Medicago truncatula*, and *Populus trichocarpa*) and four monocot species (*Zea mays*, *Oryza sativa*, *Brachypodium distachyon*, and *Sorghum bicolor*). The subcellular localization of each subfamily (*black*, plastids; *red*, mitochondria) is based either on experimental evidence or, in the absence of experimental evidence, on TargetP prediction. Bootstrap values are also indicated. Underlined ABC1Ks are in PGs; the boxed ABC1K13 complements the yeast ABC1 loss-of-function mutant. Abbreviations: ABC1K, ACTIVITY OF BC1 COMPLEX KINASE; PG, plastoglobule. Adapted from Reference 89.

family in algae and plants likely derived from the cyanobacterial endosymbiont and underwent several duplications after endosymbiosis (75) (see Section 8).

Several studies have reported PGs in cyanobacteria (in which they are often called lipid particles or lipid droplets) (106, 146), but their proteome and metabolome compositions are not known. High-resolution three-dimensional reconstruction of the cyanobacterium *Synechocystis* sp. PCC 6803 showed that lipid particles were abundant and that their distribution was restricted to thylakoids (146), similar to those of *Synechococcus* sp. PCC 7002 (103). Their intracellular location suggests a role in thylakoid maintenance or thylakoid biogenesis, similar to plastid PGs. The widespread presence of homologs of PG core proteins in cyanobacteria suggests that PGs or related membrane microdomains exist in cyanobacteria. Gene knockouts of the two *Synechocystis* sp. PCC 6803 FBN homologs resulted in a light-sensitive phenotype, suggesting a role in photoprotection or repair (22). However, in this particular study, no PGs were observed, and the authors therefore hypothesized that the FBNs may be recruited to specialized membrane domains rather than to PGs.

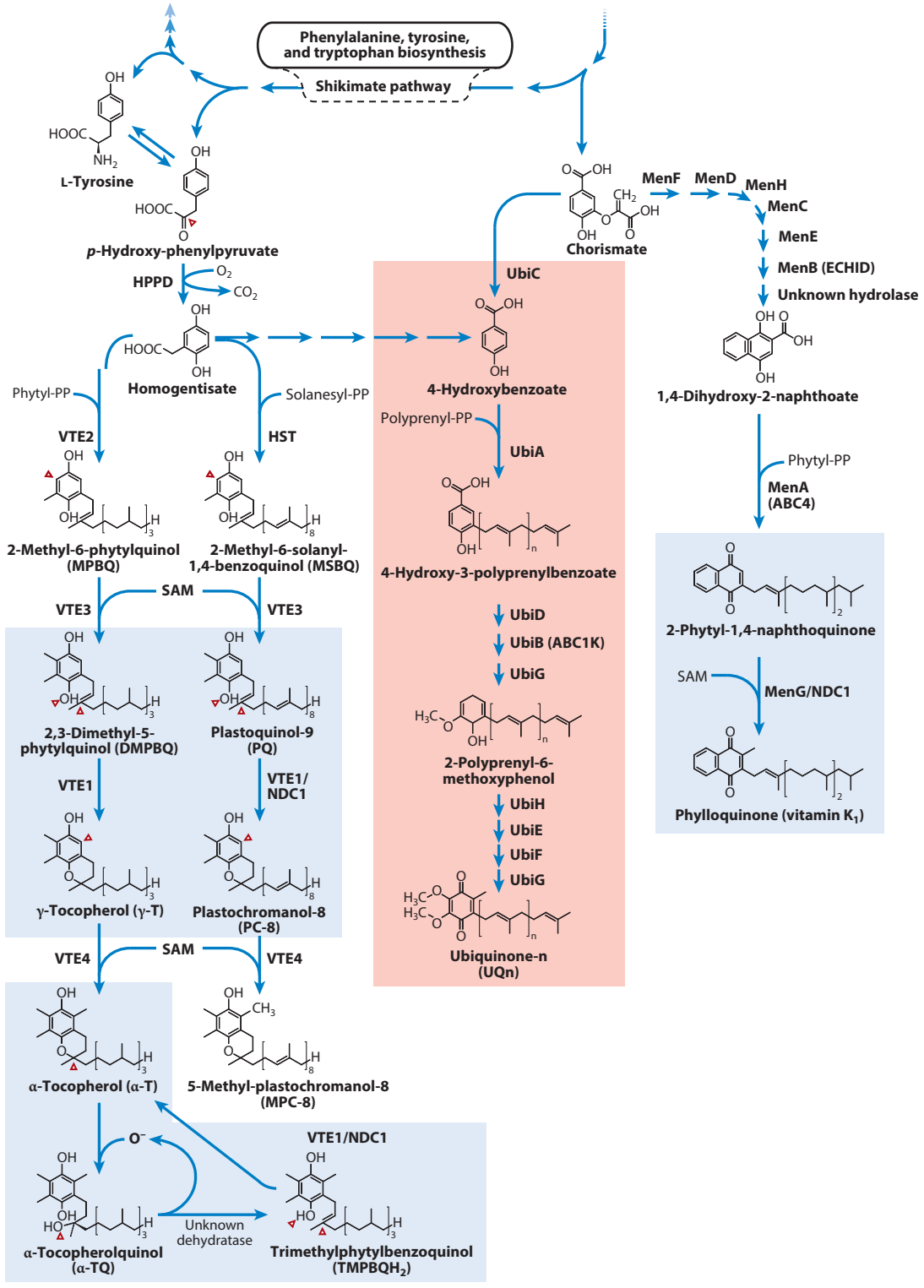
Cryoelectron tomography of *C. reinhardtii* cells identified PGs (~65-nm diameter) closely associated with thylakoid membranes, with infrequent punctate contact sites between thylakoids and PGs (32). The small-molecule and proteome compositions of *Chlamydomonas* PGs are unknown, but the *Chlamydomonas* genome encodes homologs of many higher-plant PG proteins, including FBN, ABC1K, and SOUL homologs. *Chlamydomonas* contains an additional PG-like structure, the carotenoid-rich orange eyespot, which senses light and initiates phototactic responses (69). Purification and mass spectrometry analysis showed that the eyespot proteome contains homologs of several higher-plant PG core proteins, such as HBP3 and ABC1K1 and -3, as well as carotenoid biosynthetic enzymes (30, 124, 152) (**Table 2**). The ABC1K6 homolog (designated EYE3) was identified in a genetic screen and is required for eyespot assembly (14). PGs have been postulated to serve as a precursor of eyespot pigment assembly (100). Comparative metabolomics and proteomics of *Chlamydomonas* eyespots and PGs should help to unravel their functional relationship.

D. bardawil is a halotolerant green alga, and unlike higher plants, it accumulates both cytoplasmic lipid droplets and specialized chloroplast PGs that can contain extremely large quantities of β-carotene with an unusual isomeric composition (24). Both types of lipid droplets have been isolated and analyzed to determine their chemical and proteome compositions (23, 24) (see **Table 2**). The PGs are rich in TAG, β-carotene, and phytoene. The proteome composition of *D. bardawil* cytoplasmic lipid droplets strongly resembles that of *Chlamydomonas* cytoplasmic lipid droplets, whereas the composition of its PGs resembles those of *Chlamydomonas* eyespots and *Arabidopsis* PGs, including homologs of FBN1, -7, and 8; VTE1; NDC1; HBPs; PESs; M48 protease; ABC1K1, -3, -5, -6, and -9; and β-carotene biosynthesis enzymes (**Table 2**). The similarity between the *D. bardawil* PGs and higher-plant PGs is striking (**Table 2**) and suggests highly conserved functions. The PGs also contain other proteins, such as the abundant CAROTENE GLOBULE PROTEIN (CGP) (62), that are not present in higher-plant PGs or *Chlamydomonas* eyespots (23). Unlike in other *Dunaliella* spp., no eyespot was detected in *D. bardawil*, and Davidi et al. (23) therefore proposed that *D. bardawil* PGs combine functions of both PGs and eyespots.

6. FUNCTIONS OF PLASTOGLOBULI IN ISOPRENOID METABOLISM

6.1. The Role of Plastoglobuli in Metabolism of Tocopherols and Quinones

The members of the plastid-localized family of prenylquinones—including PQ-9, phylloQ, PC-8, α-tocopherol, and α-tocopherolquinol (**Table 1**)—play important roles as electron carriers in photosynthetic electron transport and lipid antioxidants. These prenylquinones are synthesized


Phenylalanine, tyrosine, and tryptophan biosynthesis
Shikimate pathway
L-Tyrosine
p-Hydroxy-phenylpyruvate
HPPD
Homogentisate
Chorismate
MenF
MenD
MenH
MenC
MenE
MenB (ECHID)
Unknown hydrolase
1,4-Dihydroxy-2-naphthoate
Phytyl-PP
MenA (ABC4)
2-Phytyl-1,4-naphthoquinone
SAM
MenG/NDC1
Phylloquinone (vitamin K1)
UbiC
4-Hydroxybenzoate
Polyprenyl-PP
UbiA
4-Hydroxy-3-polyprenylbenzoate
UbiD
UbiB (ABC1K)
UbiG
2-Polyprenyl-6-methoxyphenol
UbiH
UbiE
UbiF
UbiG
Ubiquinone-n (UQn)
Phytyl-PP
Solanesyl-PP
VTE2
HST
2-Methyl-6-phytylquinol (MPBQ)
2-Methyl-6-solanyl-1,4-benzoquinol (MSBQ)
SAM
VTE3
VTE3
2,3-Dimethyl-5-phytylquinol (DMPBQ)
Plastoquinol-9 (PQ)
VTE1
VTE1/ NDC1
γ-Tocopherol (γ-T)
Plastochromanol-8 (PC-8)
SAM
VTE4
VTE4
α-Tocopherol (α-T)
5-Methyl-plastochromanol-8 (MPC-8)
O⁻
VTE1/NDC1
α-Tocopherolquinol (α-TQ)
Unknown dehydratase
Trimethylphytylbenzoquinol (TMPBQH2)

in an interconnected metabolic network downstream of the plastid-localized shikimate and 2-*C*-methyl-D-erythritol 4-phosphate (MEP) pathways (**Figure 4**). PQ-9 is best known for transferring electrons from photosystem II to the cytochrome b_6f complex, but PQ-9 also acts to protect the thylakoid membrane lipids from oxidation by reactive oxygen species (ROS) (71–73). Indeed, overexpression of stromal SOLANESYL DIPHOSPHATE SYNTHASE 1 (SPS1) resulted in strongly increased resistance to ROS stress, which was attributed to increased PQ-9 biosynthesis (73). A large fraction (~50%) of PQ-9 is located in PGs and not directly involved in electron transport reactions and therefore constitutes a nonphotoactive pool (73, 139). PhylloQ is required for electron transport within photosystem I and is not known to function as a lipid antioxidant. α-Tocopherol functions as a lipid antioxidant and is oxidized to α-tocopherolquinol, which can be converted back into α-tocopherol through a repair cycle involving VTE1. PC-8, the chromanol derivative of PQ-9, also acts as an antioxidant in leaves and seeds (99, 139). All five of these prenylquinones can be present in chloroplast PGs, and PQ-9 and tocopherol also accumulate in chromoplast PGs (**Table 1**). Two key enzymes in their biosynthesis and in redox recycling and repair, VTE1 and NDC1, are localized in PGs (**Figures 2**, **4**, and **5**; **Table 2**).

All enzymes of the tocopherol biosynthetic pathway—VTE1, HOMOGENTISATE PHYTYLTRANSFERASE [VITAMIN E DEFICIENT 2 (VTE2)], TOCOPHEROL METHYLTRANSFERASE [VITAMIN E DEFICIENT 3 (VTE3)], γ-TOCOPHEROL METHYLTRANSFERASE [VITAMIN E DEFICIENT 4 (VTE4)], and homogentisate prenyltransferase (**Figure 2**)—were originally thought to be located entirely at the chloroplast inner envelope membrane (see references in 163), and the discovery of VTE1 in the PG proteome was therefore surprising (148, 162). VTE1 converts 2,3-dimethyl-5-phytylquinol (DMPBQ) into γ-tocopherol and converts PQ-9 into PC-8 (108). Immunoblotting suggests that, in chloroplasts, VTE1 is present in envelope membranes, where it participates in de novo synthesis, but the majority of VTE1 is present in PGs (90), where it participates in PC8 synthesis and recycling of oxidized tocopherols. Surprisingly, the VTE1 substrate DMPBQ (**Figure 4**) is converted to tocopherol even when VTE1 is expressed in the endoplasmic reticulum (98). Several recent studies have suggested that VTE1 activity may be regulated at the posttranslational level by phosphorylation through the PG core proteins ABC1K1 and -3, but there is no direct evidence (91, 95, 96) (see Section 7).

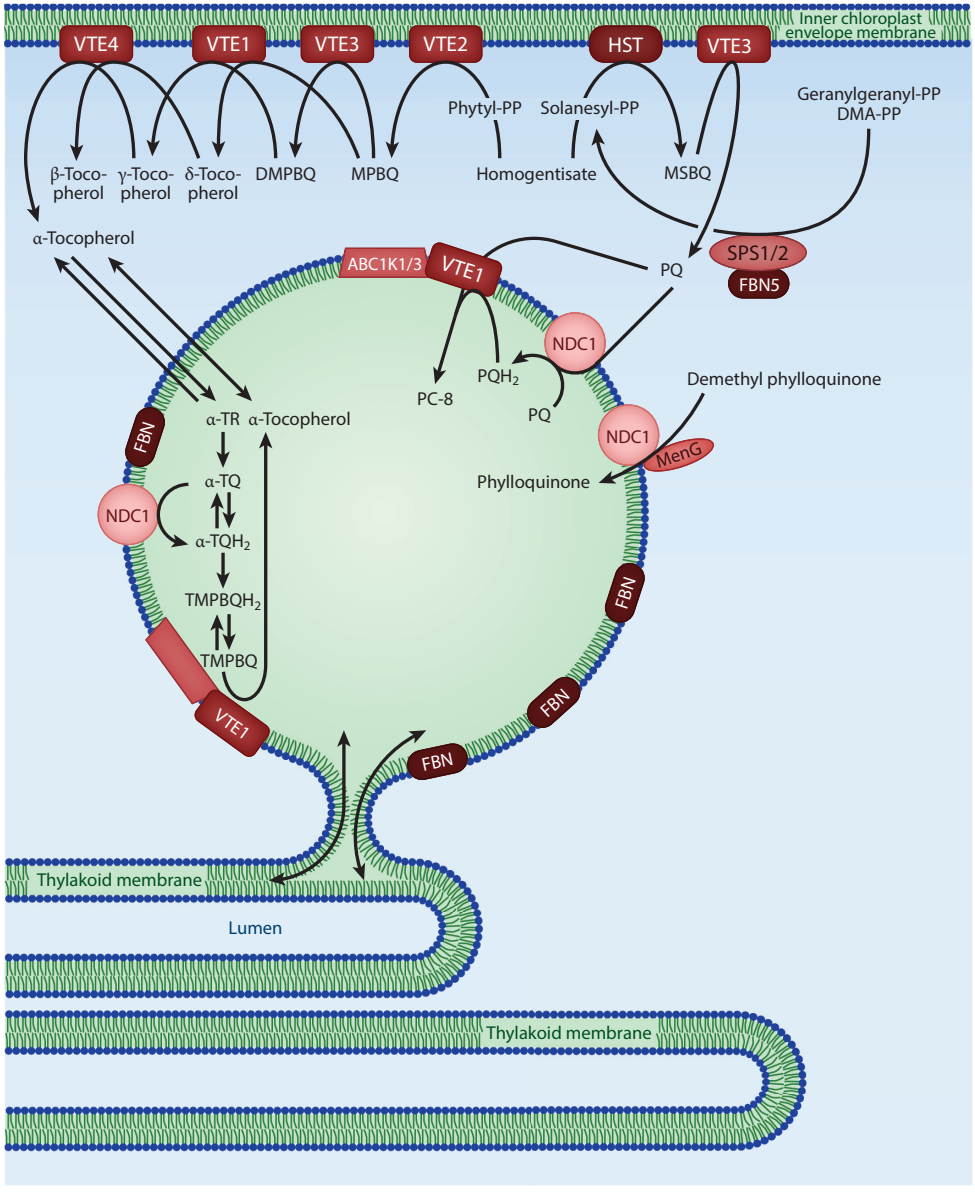
PG-localized VTE1 also functions in tocopherol redox cycling (95) (lower left corner of **Figure 4**). The scavenging of lipid peroxyl radicals by α-tocopherol results in the formation of the tocopherol oxidation product α-tocopherolquinol. Indeed, α-tocopherolquinol levels increase under high light intensity and rapidly decrease in the dark (68). [^{14}C] α-tocopherolquinol is rapidly converted to α-tocopherol in isolated chloroplasts, supporting the tocopherol recycling pathway. α-Tocopherolquinol is enriched three- to fivefold in PGs compared with thylakoids (33), consistent with a function of PGs as a tocopherol recycling compartment. The repair

Figure 4

Prenylquinone pathways: the biosynthetic pathways of tocopherol, plastoquinol, plastochromanol, ubiquinone, and phylloquinone in *Arabidopsis*. The modifications introduced in each enzymatic step are indicated with red triangles. Reactions highlighted in pale blue implicate PGs, and reactions highlighted in pale red occur in mitochondria. Abbreviations: ABC1K, ACTIVITY OF BC1 COMPLEX KINASE; ABC4, ABERRANT CHLOROPLAST DEVELOPMENT 4; ECHID, enoyl–coenzyme A hydratase/isomerase D; HPPD, *p*-hydroxy-phenylpyruvate dioxygenase; HST, homogentisate prenyltransferase; Men, menaquinone deficient; NDC1, NADP(H) DEHYDROGENASE C1; PG, plastoglobule; PP, pyrophosphate; SAM, *S*-adenosylmethionine; Ubi, ubiquinone deficient; VTE1, TOCOPHEROL CYCLASE (VITAMIN E DEFICIENT 1); VTE2, HOMOGENTISATE PHYTYLTRANSFERASE (VITAMIN E DEFICIENT 2); VTE3, TOCOPHEROL METHYLTRANSFERASE (VITAMIN E DEFICIENT 3); VTE4, γ-TOCOPHEROL METHYLTRANSFERASE (VITAMIN E DEFICIENT 4).

cycle also requires a dehydratase (68) (**Figure 4**); this postulated dehydratase could be one of the uncharacterized PG core reductases (**Table 2**).

NDC1 belongs to the family of type II NADPH dehydrogenases or NAD(P)H:quinone oxidoreductases (31, 101). It is conserved from cyanobacteria, and in *Arabidopsis* it is the only one of the seven family members localized in chloroplasts (19). It is localized in the PGs, and in *Arabidopsis* it is essential in the penultimate step of phylloQ synthesis (**Figures 4** and **5**). Specifically, NDC1 catalyzes the reduction of demethyl phylloQ (also called 2-phytyl-1,4-naphthoquinone), a prerequisite for its methylation by demethylmenaquinone methyltransferase (MenG; At1g23360), the final enzyme in phylloQ synthesis (35, 87) (**Figure 4**). NDC1 has a second function in



reducing PQ-9 to PQ-9-H_2 in the PG-localized, nonphotoactive PQ-9 pool (33), which affects the overall PQ-9 redox state. Thus, it has been proposed that NDC1 in PGs conditions the lipid medium for reactions that favor reducing conditions, i.e., phylloQ biosynthesis, PC-8 formation, and tocopherol recycling.

6.2. The Role of Plastoglobuli in Carotenoid Metabolism in Chloroplasts and Chromoplasts

PGs in chromoplasts accumulate high levels of carotenoids and carotenoid esters (**Table 1**), giving chromoplasts their hallmark orange to red colors. As discussed in Section 2, chromoplast PGs often have tubular or fibrillar structures (**Figure 1**). During fruit ripening and chromoplastogenesis, carotenoid levels increase concomitantly with changes in PG size and shape and loss of thylakoid membranes (see Section 3). Chromoplast PGs are highly enriched for several FBNs (**Table 2**), particularly FBN1a and -1b, as well as the carotenoid biosynthetic enzymes ZDS (involved in the synthesis of all-*trans*-lycopene) and LYC-β (involved in the cyclization reactions that generate carotene) (**Figure 6**, **Table 1**). These enzymes lack transmembrane domains and would therefore be able to associate with PGs. The clustering of these enzymes at the PGs and associated membranes may facilitate substrate channeling and accumulation of carotenoids inside PGs and fibrils.

Pepper chromoplast PGs also contain an esterase (162) (**Table 2**) that is closely related to *Arabidopsis* PES1 (83) and tomato PALE YELLOW PETAL 1 (PYP1) (2). Thus, the PES1/PYP1 homologs in chromoplast PGs probably play a key role in carotenoid esterification (**Figures 6** and **7**). PES1 was also observed in PGs of chloroplasts (**Table 2**), and *PES1* mRNA expression in leaves was strongly induced during senescence (90). Furthermore, carotenoid levels in the PGs of chloroplasts were very low but transiently increased in PGs during senescence concomitantly with the loss of carotenoids from thylakoid membranes (140) (**Table 1**).

Chloroplast and chromoplast PGs contain an additional carotenoid metabolic enzyme, CCD4 (90, 148, 162) (**Figure 6**, **Table 2**). The PG core protein CCD4 and its homologs in various plant species are involved in carotenoid cleavage, in particular at the 9,10 and 9′,10′ positions, resulting in one or more apocarotenoids, especially β-ionone (54, 118) (**Figure 8**). *Arabidopsis* CCD4 is implicated in the oxidative cleavage of early carotenoid intermediates (i.e., upstream of ZDS)

Figure 5

A chloroplast PG. Chloroplast PGs are sites of prenylquinone metabolism and storage. VTE1 prefers reduced substrates for the biosynthesis of tocopherol and plastochromanol as well as tocopherol recycling. NDC1 provides the reduced substrates PQH_2 and TMPBQ for PC-8 biosynthesis and tocopherol recycling, respectively. NDC1 also catalyzes the reduction of demethyl phylloquinone prior to methylation by MenG. ABC1K1 promotes increased tocopherol production under high light, and ABC1K3 is required for normal PC-8 accumulation and tocopherol recycling; these kinases may directly act on VTE1 activity, possibly by phosphorylation. FBNs are believed to function as structural proteins. The FBN5 homolog occurs in the stroma and is required for the activity of SPS1 and -2. Abbreviations: ABC1K, ACTIVITY OF BC1-LIKE KINASE; DMA-PP, dimethylallyl pyrophosphate; DMPBQ, 2,3-dimethyl-5-phytylquinol; FBN, FIBRILLIN; HST, homogentisate prenyltransferase; MenG, demethylmenaquinone methyltransferase; MPBQ, 2-methyl-6-phytylquinol; MSBQ, 2-methyl-6-solanyl-1,4-benzoquinol; NDC1, NADP(H) DEHYDROGENASE C1; PC-8, plastochromanol-8; PG, plastoglobule; PP, pyrophosphate; PQ, plastoquinone-9; PQH_2, plastoquinol-9; SPS, SOLANESYL DIPHOSPHATE SYNTHASE; TMPBQ, trimethylphytylbenzoquinone; $TMPBQH_2$, trimethylphytylbenzoquinol; α-TQ, α-tocopherolquinone; α-TQH_2, α-tocopherolquinol; α-TR, α-tocopheroxyl radical; VTE1, TOCOPHEROL CYCLASE (VITAMIN E DEFICIENT 1); VTE2, HOMOGENTISATE PHYTYLTRANSFERASE (VITAMIN E DEFICIENT 2); VTE3, TOCOPHEROL METHYLTRANSFERASE (VITAMIN E DEFICIENT 3); VTE4, γ-TOCOPHEROL METHYLTRANSFERASE (VITAMIN E DEFICIENT 4).

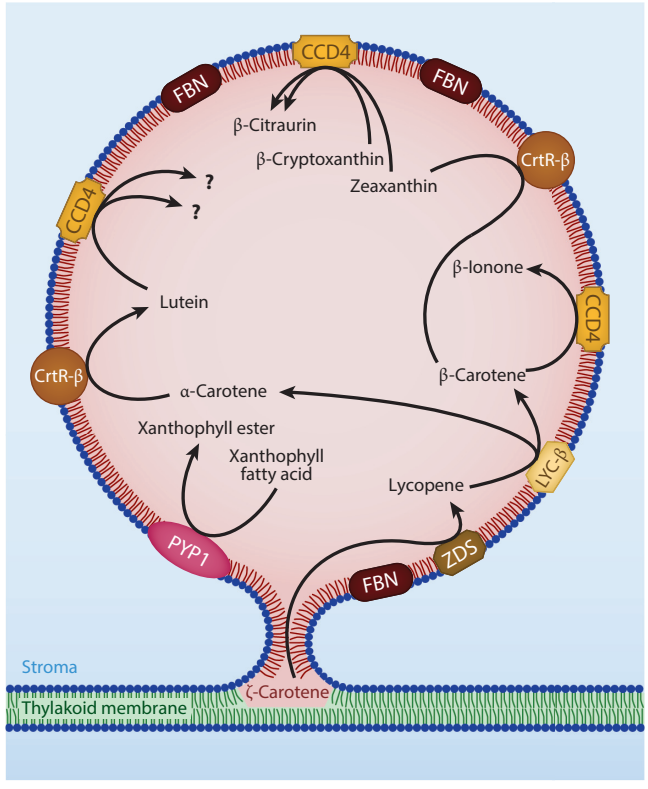


Figure 6

A chromoplast PG. Chromoplast PGs specialize in carotenoid metabolism. CrtR-β, LYC-β, and ZDS are recruited and support carotenoid biosynthesis and accumulation in PGs. CCD4 cleaves carotenoids, thereby contributing to fruit chromoplast pigmentation and volatile emission. PYP1, a PES homolog, participates in carotenoid ester synthesis in tomato petal chromoplasts. Abbreviations: CCD4, CAROTENOID CLEAVAGE DIOXYGENASE 4; CrtR-β, β-carotene β-hydroxylase; FBN, FIBRILLIN; LYC-β, lycopene β-cyclase; PES, PHYTOL ESTER SYNTHASE; PG, plastoglobule; PYP1, PALE YELLOW PETAL 1; ZDS, ζ-carotene desaturase.

that function in a signaling cascade leading to inhibition of chloroplast and leaf development in *Arabidopsis* (5). An *Arabidopsis* genome-wide association study identified CCD4 as a major negative regulator of seed carotenoid content (42); *ccd4* loss-of-function mutants exhibited increased β-carotene content upon seed desiccation and much higher carotenoid levels than wild-type plants after dark-induced leaf senescence, resulting in a yellow phenotype. RNAi-mediated suppression of the *CCD4* homolog *CCD4a* converted white petals of *Chrysanthemum* into yellow petals, indicating that CCD4a cleaves carotenoids into colorless compounds (166). Rubio et al. (118) reported that a CCD4 homolog in mandarin fruit is involved in cleavage of β-cryptoxanthin and zeaxanthin to yield a red pigment, β-citraurin (**Figure 8**).

Saffron consists of the desiccated stigma of *Crocus sativus*. It is characterized by the presence of several apocarotenoids, including crocetin, picrocosin, safranal, and β-ionone, which contribute to its color, flavor, and aroma. Two CCD4 isoforms, CsCCD4a and CsCCD4b, are expressed in the *Crocus sativus* stigma (118), and their expression correlates with the increase in PG size and number during *Crocus sativus* stigmata development, suggesting tight coordination with substrate availability in PGs. Expression of CsCCD4 in *Escherichia coli* cells engineered to accumulate carotenoids

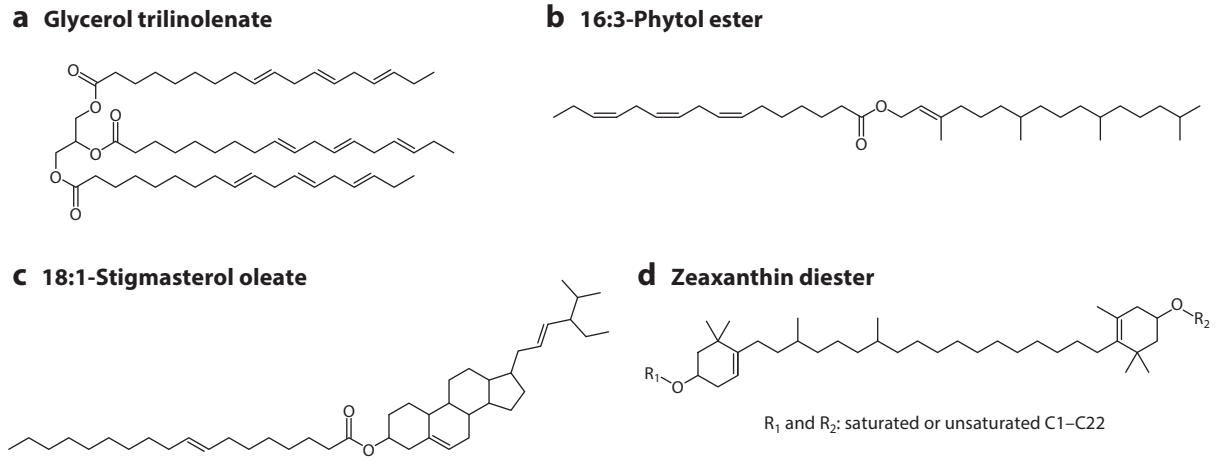


Figure 7

PES products. PES is a polyvalent enzyme that produces a variety of fatty acid esters in diverse types of PGs; shown here are generic examples of products synthesized by PES or its homologs. (*a*) A triacylglycerol species: glycerol trilinolenate produced in gerontoplast PGs. (*b*) A fatty acid phytyl ester: 16:3-phytol ester produced in gerontoplast PGs. (*c*) A sterol ester: 18:1-stigmasterol oleate produced in stigma leucoplast PGs. (*d*) A carotenoid ester: zeaxanthin diester (with the fatty acid chains indicated as R_1 and R_2), a major component of chromoplast PGs and carotenoid fibrils. Abbreviations: PES, PHYTOL ESTER SYNTHASE; PG, plastoglobule.

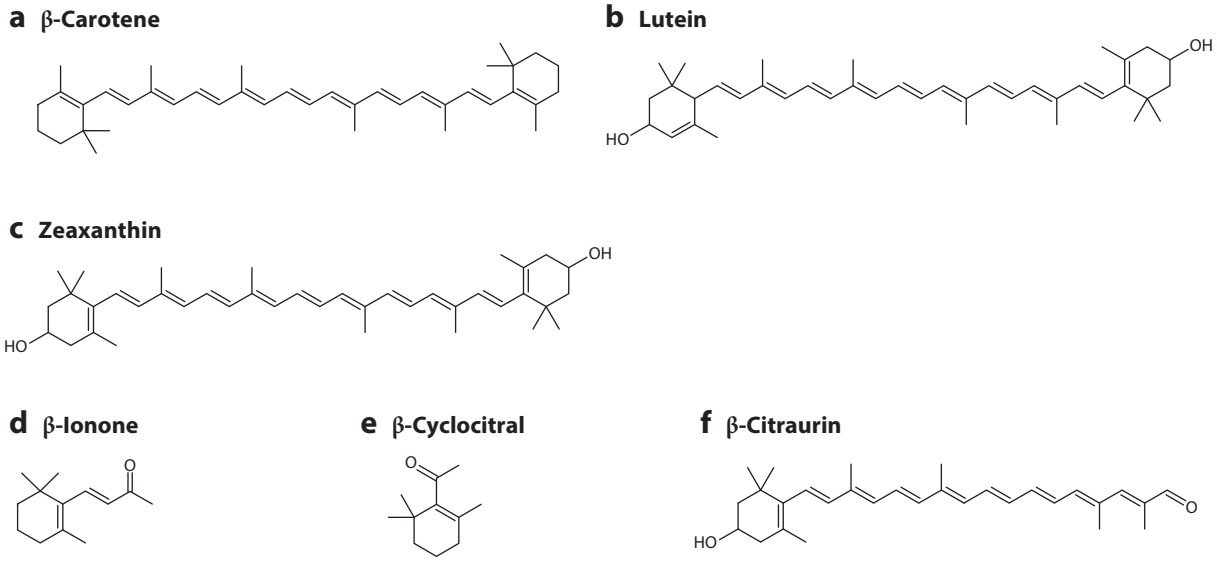


Figure 8

CCD4 substrates and products. (*a–c*) β-Carotene, lutein, and zeaxanthin, the principal substrates of CCD4. (*d*) β-Ionone, a volatile cleavage product of β-carotene that contributes to the flower scent in rose. (*e*) β-Cyclocitral, a cleavage product of β-carotene that functions as a stress signal in response to high light and affects the expression of a large set of genes in *Arabidopsis*. It may also be produced spontaneously upon reaction of β-carotene with singlet oxygen. (*f*) β-Citraurin, a reddish cleavage product of zeaxanthin or β-cryptoxanthin that colors the satsuma mandarin (*Citrus unshiu*). Abbreviation: CCD4, CAROTENOID CLEAVAGE DIOXYGENASE 4.

showed that CsCCD4 preferentially cleaves β-carotene into β-ionone and β-cyclocitral (**Figure 8**), indicating that PGs participate in flavor and volatile production in saffron (118).

7. PLASTOGLOBULI ARE INVOLVED IN CHLOROPHYLL AND LIPID BREAKDOWN

PGs supersize during senescence and nitrogen deprivation concomitantly with the disassembly of the thylakoid membranes and protein complexes and the release and breakdown of cofactors and pigments, including carotenoids and chlorophyll (**Figure 1**). Following removal of Mg^{2+} from the porphyrin ring, phytol is released by pheophytin pheophorbide hydrolase (PPH) (52, 121), and lipases release free fatty acids from the thylakoid galactolipids (64, 142). Esterification of phytol with these free fatty acids results in highly hydrophobic fatty acid phytyl esters (**Figures 2**, **7**, and **9**). In addition, thylakoid diacylglycerol (DAG) and free fatty acids may combine to form TAG (**Figure 7**). Fatty acid phytyl esters and TAG accumulate in chloroplast PGs during senescence and nitrogen deprivation (**Table 1**). PG-localized PES1 and -2 play a key role in the esterification processes (**Figure 7**). Interestingly, PPH was also observed in the PGs of senescing tissue (91), allowing direct coupling of phytol release to the production of fatty acid phytyl esters. Moreover, a protein potentially involved in removing Mg^{2+} from the porphyrin ring (AT5G17450), immediately upstream of PPH, is also located in PGs (90).

Both PES1 and -2 are strongly upregulated during senescence and during nitrogen deprivation (**Figure 9**). PES1 and -2 contain both hydrolase and acyltransferase domains. They belong to the plant ELT family and have similarity to DAG acyltransferases (83). PES1 and -2 are able to accept monogalactosyldiacylglycerol as a substrate for TAG synthesis and are also able to release fatty acids from monogalactosyldiacylglycerol (83). When expressed in a yeast mutant lacking TAG and sterol ester synthesis activity, both PES1 and -2 led to the accumulation of TAG and sterol esters, demonstrating the multifunctional nature of the two enzymes. In *Arabidopsis pes1 pes2* double mutants, fatty acid phytyl esters decreased by 80% under nitrogen deprivation, and total TAG content decreased by ~30%. A third member of the ELT family, ELT4, detected in the PG proteome (90) (**Table 2**) may also contribute to the synthesis of both fatty acid phytyl esters and TAG under nitrogen deprivation.

Lundquist et al. (91) recently showed that several plastid-localized enzymes [LIPOXYGENASE 2 (LOX2), LOX3, and LOX4; allene oxide cyclase (AOC); and allene oxide synthase (AOS)] involved in the synthesis of the plant hormone jasmonate are recruited to PGs in the *abc1k1 abc1k3* double mutant, which is deficient in two of the PG-localized ABC1Ks (**Figure 9**, **Table 2**). The LOX proteins oxidize 16:3 and 18:3 fatty acids, which are then converted into 12-oxo-phytodienoic acid (OPDA) by AOC and AOS. OPDA is then transported to the peroxisome, where β-oxidations yield the hormone jasmonate. AOS was reported in the early versions of the PG proteome (148, 162), but its widespread distribution in chloroplast membrane fractions precluded its assignment as a PG protein (90). Youssef et al. (161) also demonstrated that PGs play a role in the jasmonate pathway in an *Arabidopsis* RNAi line with reduced levels of FBN1a, -1b, and -2 (see Section 8). The data suggest that the PGs function as a site of initiation for jasmonate biosynthesis, particularly during stress, and that this process involves recycling of fatty acids from thylakoid lipids rather than from de novo fatty acid biosynthesis.

8. THE CONTRIBUTION OF FIBRILLINS TO PLASTOGLOBULE FUNCTION AND ORGANIZATION

The FBN family in plants and algae can be grouped into 12 clades (129). Most cyanobacterial genomes have only one or two FBN proteins, which are most closely related to FBN1 and -2. The

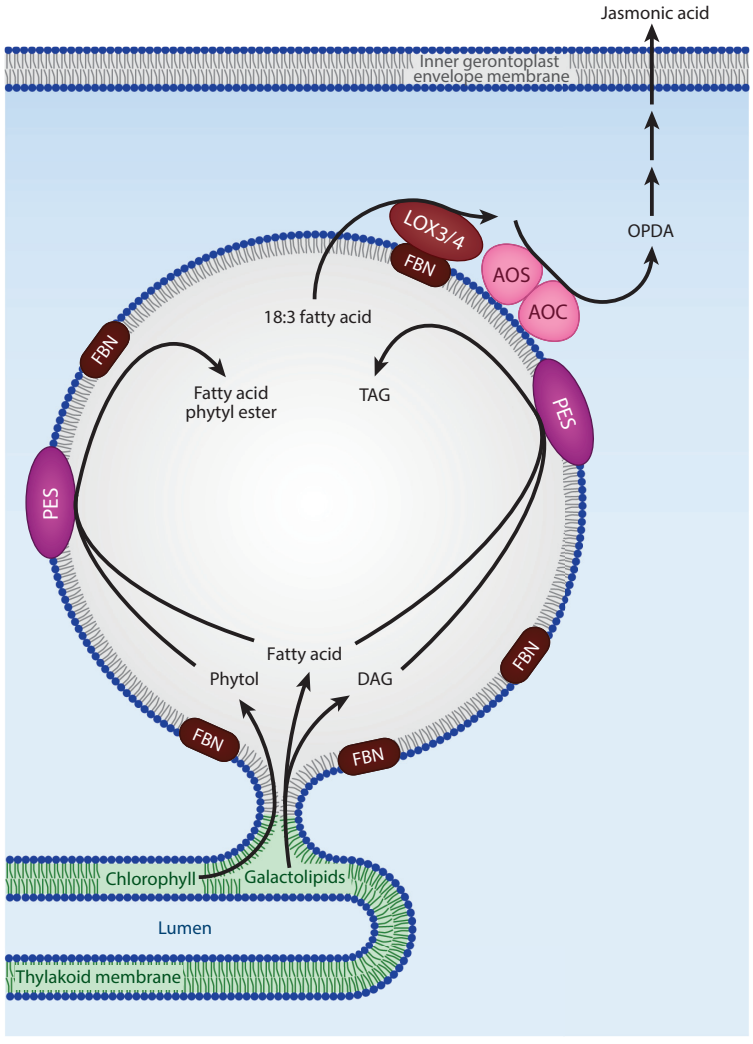


Figure 9

A gerontoplast PG. Gerontoplast PGs participate in thylakoid disassembly, galactolipid hydrolysis, and chlorophyll catabolism during senescence. Free phytol is released from chlorophyll. PES1 and -2 combine phytol and free fatty acids derived from galactolipid hydrolysis into fatty acid phytyl esters. PES1 and -2 also synthesize TAG from free fatty acids and DAG. During senescence, gerontoplast PGs recruit enzymes of the jasmonate pathway (LOX3 and -4, AOC, and AOS), contributing to thylakoid disassembly and jasmonate production. Abbreviations: AOC, allene oxide cyclase; AOS, allene oxide synthase; DAG, diacylglycerol; FBN, FIBRILLIN; LOX, LIPOXYGENASE; OPDA, 12-oxo-phytodienoic acid; PES, PHYTOL ESTER SYNTHASE; PG, plastoglobule; TAG, triacylglycerol.

origin of the plant and algal FBN proteins is not entirely clear, but they have been suggested to derive from the original cyanobacteria symbiotic and plastid progenitor (22, 75). The *Arabidopsis* genome encodes 14 FBN proteins (75, 90, 129). *Arabidopsis* PGs contain 7 FBN proteins (FBN1a, -1b, -2, -4, -7a, -7b, and -8), with the remainder of the PG proteins located in the stroma (FBN5) (66) or plastid membranes, most likely the thylakoids (90) (**Table 2**). The combined isoelectric

point and hydrophobicity of FBNs correlate with their intrachloroplast localization (90). Targeting of FBNs to PGs does not appear to involve a specific sequence within the protein; rather, it relies on the complete structure of the protein (149). FBN11 is the most distant member of the FBN family because it has a high-confidence protein kinase domain and a much higher molecular mass (76 kDa) compared with the rest of the FBN family (25–45 kDa).

The plant FBN family is diverse but shows sequence conservation in the N- and C-terminal regions, including a lipocalin(-like) signature. Lipocalins are characterized by a compact, stable, eight-stranded, antiparallel β-barrel structure enclosing an internal ligand-binding site that interacts with a variety of small hydrophobic molecules, including steroids, bilins, retinoids, and lipids (20, 122). *Arabidopsis* FBN5, which is not a PG protein but instead is a soluble stromal protein (90), is essential for PQ-9 biosynthesis because it binds to SPS1 and -2, which synthesizes the solanesyl moiety of PQ-9 (66). PGs isolated from leaves of an apple (*Malus domestica*) *fbn4* mutant with reduced FBN4 levels contained only ~10% of wild-type PQ-9 levels, whereas the overall PQ-9 levels in leaves and chloroplasts remained unchanged (127, 128), suggesting that FBN4 and its lipocalin motif function in the binding and transport of PQ-9. Three additional chloroplast lipocalins (or lipocalin-like proteins) outside of the FBN family have also been characterized: the xanthophyll cycle enzyme ZEAXANTHIN EPOXIDASE (ZEP); the lumenal enzyme VIOLAXANTHIN DEEPOXIDASE (VDE) (3, 16, 47); and the thylakoid enzyme CHLOROPLAST LIPOCALIN (CHL) (AT3G47860), which is needed for protection of thylakoid lipids against ROS molecules but has no known molecular interactors (13, 78). With the exception of CHL, these lipocalin-containing proteins all appear to interact with isoprenyl lipids (xanthophyll or PQ-9); it is therefore tempting to speculate that the other FBN members also interact with isoprenoid-derived molecules.

Reconstitution experiments with recombinant red pepper FBN1a combined with carotenoids, polar lipids, and xanthophyll diesters (the most abundant components of the carotenoid fibrils) efficiently formed PG fibrils, leading to the conclusion that (some) FBNs function as structural PG proteins (26). Overexpression of an FBN1a homolog in tobacco resulted in enlarged and more numerous PGs, which was also taken as evidence for a structural role (113, 125). Overexpression of the pepper FBN1a homolog in tomato fruit resulted in the delayed loss of thylakoids in differentiating chromoplasts, leading to the transient formation of plastids exhibiting a typical chromoplastic zone adjacent to a protected chloroplastic zone with preserved thylakoids (126); however, it is not clear what this implies with regard to FBN1a function.

mRNA-based expression studies of the FBN family and loss-of-function mutants for FBN1a, -1b, -2, and -4 in *Arabidopsis* and other species suggested the (direct or indirect) involvement of these FBNs in responses to biotic stresses (76, 77, 128), drought (34, 67, 76, 112), low temperature (75), and excess light (160). Overexpression of FBN1a in tobacco resulted in increased resistance to high-light stress (113). FBN1a is involved in abscisic acid–mediated protection from photoinhibition (160), and FBN1a, -1b, and -2 condition jasmonate production during low-temperature-induced photooxidative stress (161). The *fib1-2* RNAi line, which has reduced levels of FBN1a, -1b, and -2, displays a stress phenotype resembling that of the jasmonate-deficient *aos* mutant (161). One interpretation of these data is that FBN1a, -1b, and -2 help recruit jasmonate biosynthetic enzymes to PGs. For a more detailed overview of these stress studies, we refer readers to the review by Singh & McNellis (129).

9. ABC1 KINASES IN PLASTOGLOBULI: EVOLUTION AND FUNCTIONAL DIVERSIFICATION

ABC1Ks are atypical kinases that are present in archaea, bacteria, and eukaryotes and proliferated from 1 or 2 members in nonphotosynthetic organisms to more than 16 members in algae and

higher plants (89). The function of ABC1K homologs is best studied in the context of ubiquinone biosynthesis in *E. coli* and in mitochondria from yeast and mammalian cells. The founding member of the ABC1K family [also called UbiB kinases or ABC1 domain–containing kinases (ADCKs)] is UbiB from *E. coli*, which is required for the aerobic biosynthesis of ubiquinone (27, 107). The yeast (*Saccharomyces cerevisiae*) mitochondrial ABC1K coenzyme Q8 (ScCOQ8) and the mammalian homologs ADCK3 and -4 are required for ubiquinone synthesis in mitochondria in their respective organisms, most likely through phosphorylation-dependent assembly of a large multi-enzyme complex responsible for ubiquinone biosynthesis (49; reviewed in 43). Several members of this enzyme complex are transiently phosphorylated by COQ8 and dephosphorylated by the type 2C serine/threonine protein phosphatase PTC7 (94). It has been challenging to demonstrate COQ8-dependent in vivo phosphorylation; it is postulated that the general lack of observed phosphorylation is due to the transient nature of phosphorylation during the assembly process (43). Stefely et al. (135) recently provided mechanistic insight into the enzymatic function of ADCK3 by determining its crystal structure and then performing mutagenesis and functional assays; this work should help to better define the activities of the ABC1K family.

The ABC1K family in photosynthetic eukaryotes can be divided into an ancestral clade (including PG-localized ABC1K9 and mitochondrial ABC1K10), which has genes originating from the common ancestor; a plastid clade (ABC1K1–8), which has genes originating from the ancestral plastid donor; and a mitochondrial clade (ABC1K11–15), which has genes originating from the ancestral mitochondrial donor (89) (**Figure 3**). *Arabidopsis* has 17 ABC1Ks, of which 8 are likely localized in mitochondria and 9 in plastids, with 6 of the latter localized in PGs (89) (**Figure 3**, **Table 2**). *Arabidopsis* mitochondrial ABC1K13 can complement the yeast ScCOQ8 mutant (18). Phylogenetic analysis showed that the ABC1Ks originated from archaea, which do not synthesize the benzoquinones (ubiquinone and PQ-9) and instead synthesize naphthoquinone for electron transport. Therefore, the original function of the ABC1K family is likely the regulation of naphthoquinone synthesis.

Through endosymbiosis of plastid and mitochondrial ancestors, plants inherited and developed pathways for the benzoquinones (which have higher redox potentials than naphthoquinones) along with the corresponding regulatory ABC1Ks (21). The requirement for additional quinolic and prenyl lipid compounds likely drove the expansion of the ABC1K family in algae and higher plants (89). Notably, the ABC1K family represents the majority of known kinases in plastids and mitochondria (9, 89, 114, 123); despite this proliferation, however, relatively little is known about this family in plants. Furthermore, no phosphatase counterparts of the ABC1Ks have been identified in PGs, but two phosphatases are located at the thylakoids: PPH1/THYLAKOID-ASSOCIATED PHOSPHATASE 38 (TAP38) (111) and PHOTOSYSTEM II CORE PHOSPHATASE (PBPC) (120).

Lohscheider et al. (88) evaluated public *Arabidopsis* phosphoproteomics data in order to identify candidate ABC1K targets and a possible ABC1K hierarchical phosphorylation network within the chloroplast PG proteome; this study provides a starting point for experimental testing of phosphorylation sites in PG proteins. In total, 16 of the 30 PG core proteins, non-PG plastid ABC1K4 and -8, and mitochondrial ABC1K13 and -14 have reported phosphorylation sites. However, careful inspection of the underlying data indicates that fewer than 45% (30 phosphorylation sites with a phosphoserine:phosphothreonine ratio of ~8:1) could be confirmed, thus supporting phosphorylation of seven PG proteins, including five FBNs (88) (**Table 2**). A triple-phosphorylated peptide was reported for PG-localized VTE1; however, this peptide (partially) maps upstream of the experimentally identified N terminus, i.e., within the anticipated chloroplast transit peptide. No convincing evidence for phosphorylation of the ABC1Ks themselves was observed, leaving the question of a possible hierarchical phosphorylation between the ABC1Ks unanswered.

The functions of PG-localized ABC1K1 and -3 (55, 91, 95, 96, 158, 159) and ABC1K7 (93) have been extensively studied in *Arabidopsis*, and the function of ABC1K3 has been studied in rice (79). Rottet et al. (117) and Spicher & Kessler (134) have recently reviewed and summarized the various growth and molecular phenotypes of knockout mutants. The key findings were that ABC1K1 and -3 form a complex (91) and that loss of their functions interferes with tocopherol metabolism, suggesting direct downregulation of VTE1 by these kinases. Moreover, a redistribution of various isoprenoids between thylakoids and PGs has been observed, and there may be a link with the redistribution of PQ-9 in the FBN4-null mutant (127) discussed above. PG-localized ABC1K7 is involved in cadmium tolerance, oxidative stress response, iron distribution, and/or lipid metabolism and in crosstalk between abscisic acid and ROS signaling, but how ABC1K7 influences all these processes is unclear (59, 92, 93). The *Chlamydomonas* homolog of ABC1K6 (EYE3), located in the eyespot (**Table 2**), is involved in the biogenesis of pigment granules, but little is known about the molecular mechanisms (14).

10. OTHER PLASTOGLOBULE CORE PROTEINS WITH UNKNOWN FUNCTIONS

The PG core proteome contains additional proteins with unknown functions (**Table 2**). These include several reductases (AT1G32220, AT2G34460, and AT1G06690) that may be involved in aerobic desaturation of fatty acid and/or tocopherol recycling, a senescence-associated protein [SENESCENCE-ASSOCIATED GENE (SAG)] with a predicted alpha/beta hydrolase domain (AT1G73750), and two proteins with no predicted functional domains (AT4G13200 and AT3G43540). **Table 2** also includes AT1G28150 and AT1G52590; these are not considered PG core proteins, because they are insufficiently enriched in PGs (90), but they were originally identified in PGs (15). A recent analysis of PGs from plants grown under various conditions again identified these two proteins in PGs [see the Plant Proteome Database (PPDB) at **http://ppdb.tc.cornell.edu**], and we therefore include them in **Table 2**. The UbiE methyltransferase proteins AT3G10130 and AT2G41040 have a predicted UbiE domain with very low E-values, and both of these proteins have been identified with high confidence in PGs (see the PPDB). It therefore seems likely that they carry out methyltransferase functions for unidentified substrates within the PGs.

Finally, mRNA-based coexpression analysis has shown that the low-abundance M48 peptidase is part of a module of senescence-associated genes, including PG-localized ABC1K7; PES1; M48 peptidase; SAG; and the coexpressors PPH and PHEOPHORBIDE A OXYGENASE (PaO), both of which are involved in chlorophyll degradation. In vitro and in vivo studies have indicated that M48 indeed functions in senescence (11).

11. PLASTOGLOBULI IN METABOLIC ENGINEERING AND AGRICULTURE

PGs act as sites of storage and metabolism for a variety of nutritionally and commercially important molecules, including vitamins E and K_1, carotenoids, and TAG, and therefore may lend themselves to the engineering of the corresponding pathways and product accumulation. In some crop species, such as tomatoes and red peppers, chromoplast PGs or the PG-related carotenoid fibrils make important contributions to fruit quality, particularly pigmentation and flavor. Because accumulation of carotenoids and carotenoid esters is linked to the presence of specific biosynthetic enzymes at chromoplast PGs, engineering of PG enzyme composition could conceivably be used to tailor carotenoid composition.

PGs may also be useful sites for overproduction of recombinant proteins in plastids, because molecular pharming applications implicating PGs may also be of interest. Shanmugabalaji et al. (125) were able to target fusions of FBN1a and vaccine candidates (hepatitis C virus core protein and human immunodeficiency virus capsid particle p24) to PGs and thylakoid membranes in transplastomic tobacco plants. Whether the expression levels and yields can be boosted to levels sufficient for commercial applications of this technology remains to be seen.

Another potential field of application of PGs is in the production of biofuel by algae and land plants, in particular in nonseed plant tissues rather than oilseeds (for discussion and references, see 28). TAG is the major component of plant oils, and plastid-localized fatty acid biosynthesis provides the fatty acids of TAG in PGs and, to a large extent, those of cytosolic lipid droplets in photosynthetic tissues (142, 143). As discussed above, particularly during nitrogen-limiting conditions, excess light, or senescence, fatty acids derived from thylakoid galactolipids are transferred to PGs, resulting in their supersizing, and are subsequently transferred to cytoplasmic lipid droplets (28, 143). Therefore, a better understanding of PGs, including the control of fatty acid and TAG metabolic flux, will help in optimizing production of TAG for biofuels.

SUMMARY POINTS

1. Plastoglobuli (PGs) are lipoprotein particles in both nonphotosynthetic plastids and chloroplasts in algae, moss, and angiosperms. They serve as microcompartments with integrated functions in plastid metabolism, developmental transitions, and environmental adaptation.
2. ACTIVITY OF BC1 COMPLEX KINASE (ABC1K) and FIBRILLIN (FBN) proteins are the most abundant proteins in chloroplast PGs. Based on the presence of a lipocalin(-like) signature in FBN members and the limited experimental information, we speculate that FBNs contribute to PG function through the binding and exchange of prenyl lipid intermediates. Phosphoproteomics studies specifically designed to investigate the PG proteome and its ABC1Ks are needed to understand phosphorylation networks in PGs.
3. PGs in chloroplasts are involved in transient storage, synthesis, and recycling of plastoquinone-9, phylloquinone, tocopherol, and plastochromanol-8, including exchange with the thylakoid membrane. The main functions of PGs in chromoplasts are the synthesis and storage of carotenoids and carotenoid esters.
4. PGs play a key role in the detoxification of the phytol released during chlorophyll degradation through esterification, controlled dismantling of the thylakoid lipid bilayer through accumulation of fatty acids and triacylglycerol, and production of jasmonate.
5. There are poorly understood functional connections between PG function and chloroplast carbon metabolism (reflected in the loss of starch accumulation in ABC1K mutants), as well as unexplored coexpression patterns between Calvin-Benson cycle enzymes and PG core proteins.
6. PGs contain a dozen proteins with unknown functions that remain to be explored.
7. There is potential for discovery of additional low-abundance PG proteins in specialized plastids and/or specific developmental states or stress conditions.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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