

Update on Chloroplasts

The Biochemical Machinery of Plastid Envelope Membranes

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Plastids are semiautonomous organelles with a wide structural and functional diversity and unique biochemical pathways. As such, they are able to transcribe and translate the information present in their own genome but are strongly dependent on imported proteins that are encoded in the nuclear genome and translated in the cytoplasm. Plastids are present in every plant cell, with very few exceptions (such as the highly specialized male sexual cells), and their structural and functional diversity reflects their role in different cell types. According to their developmental stage, we distinguish them as juvenile (proplastids), differentiating, mature, and senescent. Meristematic cells contain proplastids, which ensure the continuity of plastids from generation to generation and are capable of considerable structural and metabolic plasticity to develop into various types of plastids that remain interconvertible. When leaves are grown in darkness, proplastids differentiate into etioplasts, which can be converted into chloroplasts under illumination.

The metabolism of these various types of plastids is linked to the function of the tissue in which they are found. For instance, whereas the chief function of illuminated leaves is the assimilation of CO₂ by chloroplasts, root plastids are mainly involved in the assimilation of inorganic nitrogen. Amyloplasts, which contain large starch grains, behave as storage reservoirs in stems, roots, and tubers. Chromoplasts synthesize large amounts of carotenoids and are present in petals, fruits, and even roots. The interconversions between these different plastids are accompanied by dramatic changes, including the development or regression of internal membrane systems (e.g. thylakoids and prolamellar bodies) and the acquisition of specific enzymatic equipment reflecting specialized metabolism. However, at all stages of these transformations, the two limiting envelope membranes remain apparently unchanged.

Located at the interface between plastids and the surrounding cytosol, the envelope is a key structure for the integration of plastid metabolism within the cell. Because plastids are semiautonomous organelles, a tight coordination between plastidial development and cell differentiation is required. Envelope membranes are an essential checkpoint between the expression of plastidial and nuclear genomes, for example, as the site for the specific

recognition and transport of the precursor plastid proteins synthesized on cytosolic ribosomes.

Plastid membranes contain an astonishing variety of specific lipids, including polar lipids (e.g. galactolipids, phospholipids, and SLs), pigments (e.g. carotenoids and chlorophylls), and prenylquinones (e.g. plastoquinone and tocopherols). This diversity requires complex metabolic pathways that are closely associated with envelope membranes.

A unique biochemical machinery (Fig. 1) is present in envelope membranes and reflects the stage of development of the plastid and the specific metabolic requirements of the various tissues.

PLASTID ENVELOPE MEMBRANES AND THE COORDINATION OF THE EXPRESSION OF NUCLEAR AND PLASTID GENOMES

Plastids rely mostly on the nucleus for their development, and the coordination between the expression of plastid and nuclear genes requires an exchange of information between the nucleus and the organelle. Envelope membranes at the border between plastids and the cytosol play a role in this coordination at least at two levels, by interacting with the plastid translation and transcription apparatus, and through the import of nuclear-encoded proteins.

In plastids cpDNA exists as large protein-DNA complexes called nucleoids, which are associated with the translation machinery. Although the replication and transcription of cpDNA are probably regulated within the plastid nucleoids, little is known about how this is accomplished or about nucleoid structure. The morphology of plastid nucleoids is subject to dynamic changes during plastid development (Sato et al., 1997). Proplastids contain a single, centrally located nucleoid. In developing plastids nucleoids move to the periphery, apparently associated with envelope membranes, and are extensively replicated. Conversely, in mature chloroplasts plastid nucleoids are dispersed within the organelle as small particles associated

Abbreviations: cpDNA, chloroplastic DNA; DGDG, digalactosyldiacylglycerol; MGDG, monogalactosyldiacylglycerol; PA, phosphatidic acid; PC, phosphatidylcholine; PG, phosphatidylglycerol; SL, sulfolipid; X:Y, a fatty acyl group containing X carbon atoms and Y *cis* double bonds.

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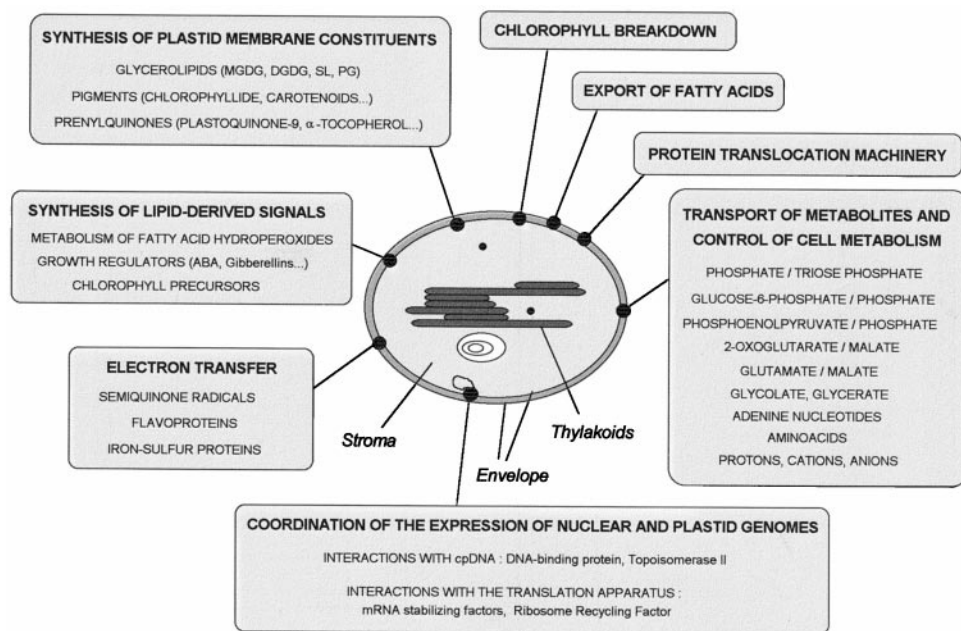


Figure 1. Functions of plastid envelope membranes.

with thylakoids. As leaves undergo senescence, the number of plastid nucleoids and the copy number of cpDNA decrease.

The inner envelope membrane of developing plastids contains a DNA-binding protein, PEND (plastid envelope DNA), which binds to several specific regions of cpDNA (Sato et al., 1993). The cDNA for PEND protein was cloned and the corresponding protein was purified and characterized. This protein contains a bZIP domain, a sextuplet repeat region, and a putative membrane-spanning region (Sato et al., 1998). Its expression is restricted to the early stages of plastid development; the PEND transcript was detected in leaf buds of 6-d-old pea seedlings but not in older ones. This is consistent with the observation that the PEND protein, as detected by its DNA-binding activity, was maximal in the envelope membranes of young plastids from 5- or 6-d-old pea seedlings (Sato et al., 1993). This suggests that PEND protein is involved in binding plastid nucleoids to the inner envelope membrane, which might affect the processes of replication, segregation, and/or transcription of cpDNA. Another protein, topoisomerase II, which is required for decatenating plastid DNA molecules after division, is also localized in the vicinity of plastid envelope membranes (Marisson and Leech, 1992).

Two observations suggest that envelope membranes could be involved in the regulation of chloroplast gene expression. First, a yellow membrane fraction derived from *Chlamydomonas reinhardtii* chloroplasts (most likely corresponding to envelope membranes) contain the stabilizing factors of some specific chloroplast mRNAs encoding thylakoid membrane proteins (Zerges and Rochaix, 1998). Second, Rolland et al. (1997) characterized a chloroplast protein homologous to a prokaryotic ribosome-recycling factor. In *Escherichia coli* this factor is essential to the termination of protein synthesis. A small proportion of this

protein was found in envelope membranes, suggesting that it could be involved in the regulation of chloroplast mRNA translation.

The import of nuclear-encoded plastid proteins across envelope membranes is another major aspect of the coordination of chloroplast and nuclear genome expression. The characterization of putative constituents involved in the envelope-import machinery is presently the most active field in envelope research. The reader is referred to a recent review by Heins et al. (1998) for a detailed presentation of the envelope-translocation apparatus and its components. It is essential to determine whether all of these components actually play a role in vivo, for example, by using genetic approaches. Finally, the evolution of the envelope-import machinery during plastid development remains to be elucidated.

PLASTID ENVELOPES AND THYLAKOIDS HAVE UNIQUE GLYCEROLIPIDS

The development of fully functional plastids depends on a complex set of envelope enzymes for the biosynthesis of specific lipid constituents of plastid membranes. Characterized by a unique glycerolipid composition (for review, see Douce and Joyard, 1996), membranes from all plastid types differ strikingly from other plant cell membranes: they contain large amounts of SLs and galactolipids (MGDG and DGDG) and few phospholipids, mostly PG. In chloroplasts this phospholipid is unique because it contains a 16:1_t (*trans*- Δ_3 -hexadecenoic acid) fatty acid at the *sn*-2 position of the glycerol backbone. PC is present only in the cytosolic leaflet of the outer envelope membrane and phosphatidylethanolamine cannot be detected in highly purified plastid membranes, including the outer envelope

membrane. In contrast, these two phospholipids are major constituents of all extraplastidial membranes.

The inner envelope membrane and the thylakoids have a very similar glycerolipid composition. Total (outer plus inner) envelope membranes from proplastids, etioplasts, and chloroplasts have an almost identical lipid composition. The lipids in the outer leaflet of the outer envelope membrane appear to have some physiological importance: their specific interaction with transit peptides affects both targeting and translocation of the precursor proteins to plastids (for review, see Heins et al., 1998).

Galactolipids, especially MGDG, are characterized by a very high content of polyunsaturated fatty acids, mostly 18:3 (linolenic acid) and, to a lesser extent, 16:3 (hexadecatrienoic acid). Plant MGDG consists of two main molecular species: the first (and major) one has 18:3 at both the *sn*-1 and *sn*-2 positions of the glycerol backbone (18:3/18:3), and the second has 18:3 at the *sn*-1 position and 16:3 exclusively at the *sn*-2 position of the glycerol (18:3/16:3). The former structure (C18/C18) is found in all eukaryotic lipids and is therefore called "eukaryotic." The latter one is similar to that of cyanobacteria glycerolipids (i.e. with C16 fatty acid at the *sn*-2 position of the glycerol, C18/C16) and is called "prokaryotic." Some plants, such as pea, contain MGDG with only the eukaryotic structure (18:3/18:3) and are therefore called "18:3" plants. Other plants, such as spinach, contain MGDG with 16:3 fatty acids and are therefore called "16:3" plants. This fatty acid is present only in prokaryotic MGDG; however, 16:3 plants usually contain both prokaryotic and eukaryotic MGDG, whereas a limited number of species (such as *Anthriscus cerefolium*) contain only prokaryotic MGDG. Most SL and plastid PG molecules also have a prokaryotic structure.

ENVELOPE MEMBRANES ARE THE SITE FOR GLYCEROLIPID BIOSYNTHESIS

As the only common membrane structure among plastids, envelope membranes contain the machinery for the assembly of plastid-specific glycerolipids, i.e. from the fatty acids, glycerol, and polar head groups (Gal for galactolipids, sulfoquinovose for SL, and glycerol for PG; Fig. 2). The biosynthesis of fatty acids occurs in the stroma, whereas the polar part of glycerolipids is made in the cytosol. Envelope membranes from chloroplasts and from nongreen plastids contain the enzymes for the acylation of *sn*-glycerol-3-phosphate to PA (Kornberg-Pricer pathway), a phospholipid at the branch point between PG and glycolipid biosynthesis. The characterization of glycerolipid biosynthetic enzymes has been hampered mostly because the purification of such membrane-bound proteins is difficult. It is difficult to use reverse genetics to obtain gene sequences, because mutations causing a deficiency in MGDG will likely be lethal. However, glycerolipid-deficient prokaryotes such as *Rhodobacter sphaeroides* and *Synechococcus* sp. pcc tg4z mutants lacking SL (Rossak et al., 1995) might be used as models for further characterization of higher plant enzymes. Our present knowledge of glycerolipid biosynthesis in envelope membranes (for review, see

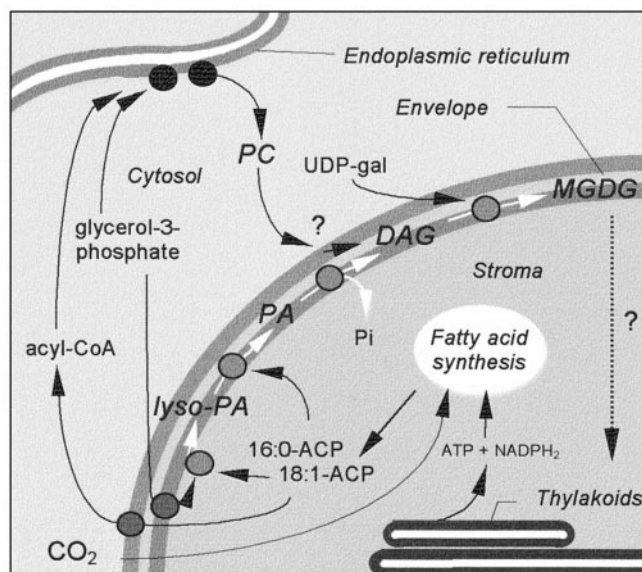


Figure 2. Biosynthesis of MGDG in a spinach chloroplast. The biosynthesis of fatty acids (under the form of 16:0-acyl-carrier protein [ACP] and 18:1-ACP) occurs in the stroma (some are exported from plastids as acyl-CoA derivatives), whereas UDP-Gal and *sn*-glycerol-3-phosphate are made in the cytosol. Plastid envelope membranes contain the enzymes for the acylation of *sn*-glycerol-3-phosphate to lyso-PA and PA, a phospholipid at the branch point between PG and glycolipid biosynthesis. In the spinach type of 16:3 plants, MGDG is made from diacylglycerol (DAG) synthesized in the envelope (prokaryotic pathway) or derived from extraplastidial glycerolipids (probably PC, which is made in the ER). Very little is known about the extraplastidial (eukaryotic) pathway and how/where PC is converted to DAG. In some 16:3 plants (such as *A. cerefolium*), only the prokaryotic pathway is active. In 18:3 plants such as pea, phosphatidate phosphatase, which converts PA into DAG, is apparently lacking and only the eukaryotic pathway seems to be functional. Very little is known about lipid transfer between membranes, i.e. DAG transfer to the inner envelope membrane, and about the transfer of newly synthesized glycerolipids such as MGDG to the outer envelope membrane and to thylakoids.

Douce and Joyard, 1996) is summarized in the following paragraphs.

Two acyltransferases catalyze the biosynthesis of PA containing 18:1 (oleic acid) and 16:0 (palmitic acid) at the *sn*-1 and *sn*-2 positions, respectively, of the glycerol moiety, i.e. with the prokaryotic structure. Therefore, the diacylglycerol synthesized from PA by the envelope phosphatidate phosphatase has the same structure. The acyltransferase responsible for lyso-PA biosynthesis is a soluble enzyme and is the only one of these enzymes for which biochemical and molecular data (and Arabidopsis mutants) are available.

MGDG synthase has been characterized on a functional basis (for review, see Douce and Joyard, 1996) and on a molecular basis (Shimajima et al., 1997). MGDG synthase competes with SL synthase for diacylglycerol but also discriminates among the different diacylglycerol molecules available in the membrane (Maréchal et al., 1994). Because MGDG synthase has a high affinity for the 18:2 (linoleate)/18:2 diacylglycerol, this eukaryotic substrate can be used

by the enzyme in addition to the prokaryotic 18:1/16:0 diacylglycerol synthesized directly within the inner envelope membrane (Maréchal et al., 1994). The kinetic properties of the envelope MGDG synthase could explain the synthesis of prokaryotic and eukaryotic MGDG molecular species. Finally, MGDG synthase is a very minor protein—about 1/1000 of the envelope proteins, which is surprising considering that MGDG is the most abundant membrane lipid on earth.

DGDG synthesis is poorly understood. MGDG and DGDG have different fatty acid compositions, and therefore most MGDG molecules cannot be incorporated directly into DGDG. The only envelope enzyme that synthesizes DGDG is a galactolipid:galactolipid galactosyltransferase that is unable to discriminate (at least in vitro) between MGDG molecular species. This enzyme is localized on the cytosolic face of the outer envelope membrane, and this topographic localization raises questions about its physiological role. Analysis of an Arabidopsis mutant (*dgd1*) showing a reduced DGDG content suggests that the galactolipid:galactolipid galactosyltransferase is not the main route for DGDG synthesis (Dörmann et al., 1995).

Chloroplasts from 18:3 plants are apparently devoid of phosphatidate phosphatase activity. Therefore, in these plants the Kornberg-Pricer pathway in the envelope is not functional and the deficiency in C18/C16 diacylglycerol synthesis is apparently compensated for by an increase in lipid synthesis in the extraplastidal compartments and by a transfer of lipids containing a C18/C18 diacylglycerol backbone to chloroplasts. The same situation probably occurs in an Arabidopsis mutant (*act1*) defective in acylation of glycerol-3-phosphate. In this mutation Arabidopsis, which is normally a 16:3 plant, is converted to an 18:3 plant. However, the mechanisms responsible for this high flexibility of lipid biosynthesis are poorly understood. A phospholipase C (not yet identified) probably generates diacylglycerol from PC, which is synthesized on the ER. Then diacylglycerol would be transferred from the outer to the inner envelope membrane, where MGDG synthesis takes place.

The lipid-transfer mechanisms between membranes, from the ER to the envelope, between the two envelope membranes, or from the inner envelope membrane to thylakoids, are almost unknown.

In plant cells most if not all 16:0, 18:0, and 18:1 fatty acids are synthesized within the plastid stroma. The mechanisms involved in fatty acid export from the stroma to the cytosol for phospholipid (e.g. PC and phosphatidylethanolamine) synthesis in the ER have not been analyzed, but the acyl-CoA synthetase localized on the outer envelope membrane is a good candidate for releasing fatty acids into the cytosolic compartment.

THE ENVELOPE IS A SITE FOR FATTY ACID DESATURATION

Fatty acids of the newly synthesized polar lipids are desaturated to form the polyunsaturated molecular species characteristic of plastid glycerolipids. The characterization of Arabidopsis mutants impaired at five loci, which are

named *fad4* to *fad8*, sheds new light on chloroplast membrane desaturases (for review, see Miquel and Browse, 1998). The *fad4* gene product is responsible for inserting a Δ_3 -*trans* double bond into the 16:0 fatty acid esterified to *sn*-2 of PG, and the *fad5* gene product is responsible for the synthesis of Δ_7 -16:1 on MGDG and possibly on DGDG. The 16(18):1 desaturase is encoded by the *fad6* gene, whereas two 16(18):2 desaturase isozymes are encoded by *fad7* and *fad8*. In contrast, very little is known about the exact localization and biochemistry of these enzymes. A plastidial n-6 desaturase was purified from chloroplast envelope membranes and the corresponding cDNA obtained by Schmidt et al. (1994).

Reduced Fd ($E'_0 = -0.4$ V) has been proposed as the electron source for O_2 reduction to H_2O ($E'_0 = -0.8$ V; for review, see Heinz, 1993), but unambiguous evidence for the involvement of reduced Fd is lacking. Since Fd delivers only one electron at a time, the desaturase must sequentially oxidize two reduced Fd molecules and store the first electron before the double bond is formed. This is possible only if electron-transfer chains exist in envelopes. Some putative components were characterized in spinach chloroplast envelope membranes: (a) semiquinone and flavosemiquinone radicals, (b) a series of iron-sulfur electron-transfer centers, and (c) flavins (mostly FAD) loosely associated with proteins (Jäger-Vottero et al., 1997). Therefore, envelope membranes probably contain all of the enzymatic constituents of a redox chain essential for fatty acid desaturation, including electron carriers involved in the formation and reduction of semiquinone radicals (quinol oxidase, NADPH-quinone, and NADPH-semiquinone reductases).

PLASTIDS CONTAIN SPECIFIC TERPENOID COMPOUNDS THAT ARE SYNTHESIZED IN ENVELOPE MEMBRANES

Chlorophyll is the most conspicuous pigment in thylakoids. In contrast, envelope membranes are devoid of chlorophyll but contain protochlorophyllide and chlorophyllide, which are barely detectable in thylakoids (for review, see Douce and Joyard, 1996). The major carotenoid in envelope membranes is violaxanthin, whereas β -carotene and lutein/zeaxanthin predominate in thylakoids. The major prenylquinone is α -tocopherol in envelope membranes and plastoquinone-9 in thylakoids. In envelope membranes molecules such as α -tocopherol and xanthophylls probably have photoprotective and stabilizing functions to maintain the integrity of membranes under the potentially harmful conditions that prevail within a photosynthesizing chloroplast. These molecules have very different chemical structures, but all derive at least in part from isopentenyl P*P*_i, a C5 isoprene unit precursor synthesized from pyruvate/glyceraldehyde 3-phosphate via a non-mevalonate 1-deoxy-D-xylulose-5-phosphate pathway (Lichtenthalen et al., 1997).

The condensation of isopentenyl P*P*_i and its isomer dimethylallyl P*P*_i, followed by the action of a series of prenyltransferases, generates geranyl P*P*_i (C₁₀), farnesyl P*P*_i (C₁₅), geranylgeranyl P*P*_i (C₂₀), and so on up to solanesyl

PPi (C₄₅) (for review, see Lichtenthaler, 1993). In plant cells terpenoid biosynthesis is a highly compartmentalized process and is not restricted to plastids (for review, see Barbier-Brygoo et al., 1997). However, within specialized tissues all plastid types synthesize a wide variety of mono-, di-, and tetraterpenes, such as monoterpenes in the leukoplasts of secretory cells and carotenoids in chromoplasts. The common part of the biosynthesis of chloroplast terpenoid compounds, beginning with isopentenyl PPi, is located within the stroma, and the final steps are associated with the inner envelope membrane (Fig. 3). The prenyltransferases involved in geranylgeranyl PPi biosynthesis are soluble and localized in the chloroplast stroma, where the products of the reaction are used by membrane-bound enzymes catalyzing a number of different reactions (e.g. prenyl and methyl transfers and cyclizations) responsible for carotenoids, prenylquinones, and chlorophyll synthesis (for review, see Douce and Joyard, 1996).

Phytoene is a C₄₀ carotenoid formed by condensation of two all-*trans*-geranylgeranyl PPi molecules. It is the precursor for all desaturated and oxygenated carotenoids. Carotenoid biosynthesis and its regulation are not as well

characterized in chloroplasts as they are in chromoplasts. Pepper and tomato chromoplasts (and also cyanobacteria) are good models with which to investigate carotenoid biosynthetic enzymes (for review, see Sandman, 1994). Substantial evidence exists for the participation of envelope membranes in carotenoid biosynthesis. Proplastids or amyloplasts contain carotenoids, and their envelope membranes (the only membrane in these organelles) could be a site of carotenoid synthesis. In addition, chromoplast membranes, which are assumed to derive from the inner envelope membrane, are very active in carotenoid biosynthesis. Finally, phytoene synthase and desaturase and zeaxanthin epoxidase activities were demonstrated in envelope membranes from spinach chloroplasts. The gene encoding zeaxanthin epoxidase from *Nicotiana plumbaginifolia* has been recently cloned (Marin et al., 1996).

Phylloquinone and α -tocopherol contain a C₂₀ phytyl chain, whereas plastoquinone-9 contains a C₄₅ solanesyl chain. In chloroplasts the inner envelope membrane is the site of α -tocopherol and plastoquinone-9 synthesis. Tocopherols are synthesized by condensation of homogentisic acid and a C₂₀-prenyl PPi to form 2-methyl-6-prenylquinol,

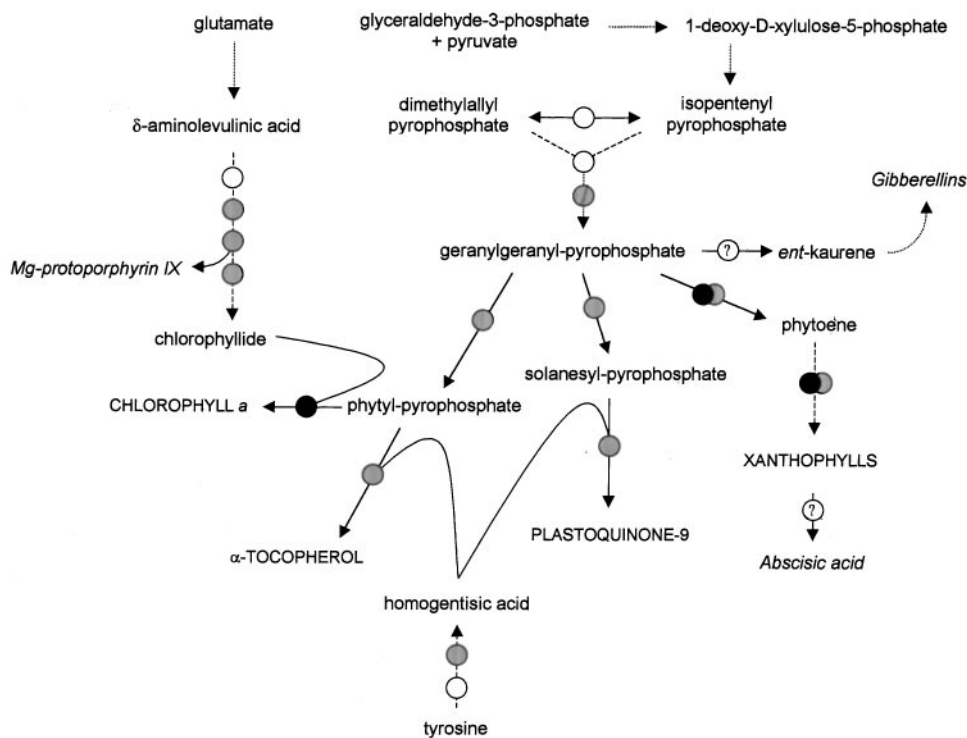


Figure 3. Compartmentation of the biosynthesis of terpenoid compounds in chloroplasts. Envelope membranes play a key role in the biosynthesis of the main plastid terpenoid compounds (prenylquinones and pigments). Geranylgeranyl-PPi, which is made from isopentenyl PPi (deriving from 1-deoxy-D-xylulose-5-phosphate, which is made from pyruvate and glyceraldehyde-3-phosphate), is the precursor for *ent*-kaurene and phytoene and for the side chains of chlorophyll, α -tocopherol, and plastoquinone-9. Envelope membranes are also the site for the biosynthesis of chlorophyll precursors (from δ -aminolevulinic acid, which is made from glutamate) and of homogentisic acid (from 4-hydroxyphenylpyruvate, which is made from Tyr). Plastid terpenoid compounds are also the precursors for several molecules involved in signaling processes, i.e. ABA (deriving from xanthophylls), GAs (deriving from *ent*-kaurene), and Mg-protoporphyrin IX. The color of the circles indicates the location of the enzymes or pathways: white for stroma, gray for envelope, and black for thylakoids. Some pathways are not yet precisely located in plastids and are indicated by “?”. Plastid constituents are indicated in capital letters, whereas molecules involved in signaling are indicated by italics.

which is then converted to 2,3-dimethyl-6-prenylquinol, γ -tocopherol, or γ -tocotrienol, and, finally, to α -tocopherol or α -tocotrienol, by a series of methylations and cyclization. Plastoquinone-9 is also synthesized in the inner envelope membrane by condensation of homogentisic acid and solanesyl-PPi to form 2-methyl-6-solanesylquinol, which is methylated and oxidized to form successively plastoquinol-9 and plastoquinone-9. Swiezewska et al. (1993) proposed that plastoquinone and ubiquinone biosynthesis was localized in Golgi membranes and that a specific transport system was required for plastoquinone and ubiquinone transfer to chloroplasts and mitochondria. However, the characterization of a nuclear-encoded methyltransferase catalyzing the last step in ubiquinone biosynthesis and localized in the inner membrane of plant mitochondria (Avelange-Macherel and Joyard, 1998) does not favor this hypothesis.

THE BIOSYNTHESIS OF CHLOROPHYLLIDE AND THE DEGRADATION OF CHLOROPHYLL ARE IMPORTANT ENVELOPE FUNCTIONS

We have shown (for review, see Douce and Joyard, 1996) that both parts of chlorophyll molecules, the porphyrin ring (i.e. chlorophyllide) and the phytyl chain, are made in envelope membranes (Fig. 3). However, the addition of phytyl PPi to chlorophyllide, a reaction catalyzed by chlorophyll synthase, is specifically associated with thylakoids. Nakayama et al. (1998) demonstrated that Mg-chelatase activity, which catalyzes the insertion of Mg^{2+} into protoporphyrin IX, was regulated by its subchloroplastic localization. The migration of Mg chelatase subunit ChlH from the stroma to envelope membranes, where it is functional, is regulated by the increase of Mg^{2+} concentration into the stroma under illumination. Two other enzymes of the chlorophyllide biosynthetic pathway, i.e. protoporphyrinogen oxidase (Matringe et al., 1992) and protochlorophyllide reductase (Pineau et al., 1986), are localized in the envelope and catalyzed by the NADPH- and light-dependent conversion of protochlorophyllide into chlorophyllide. The import of the precursor for protochlorophyllide reductase into chloroplasts requires protochlorophyllide (Reinbothe et al., 1995).

Recently, Matile et al. (1996) focused our attention on the breakdown of plant pigments in senescent leaves. They characterized chlorophyll catabolites, proposed a pathway for chlorophyll breakdown in senescing plastids (gerontoplasts), and characterized a series of enzymes (e.g. chlorophyllase and Mg-dechelatease) in the inner envelope membrane. Because chlorophyll can induce photoxidative damages, its breakdown in envelope membranes can be regarded as a process of detoxification crucial for the viability of senescent mesophyll cells. Therefore, enzymes involved in chlorophyll synthesis and degradation are present in the same membrane but at different stages of plastid differentiation, thus demonstrating the transformation of the envelope biochemical machinery during plastid interconversions.

ENVELOPE MEMBRANES AND THE SYNTHESIS OF LIPID-DERIVED SIGNALING MOLECULES FOR DEVELOPMENT AND PLANT DEFENSE

Oxylipins are a family of plant-growth regulators and defense compounds (for review, see Vick, 1993). Unlike other plant hormones, oxylipins are not stored but are synthesized and released rapidly in response to extracellular stimuli. Some are volatile, making their study difficult. The transient biosynthesis of oxylipins can be viewed as a lipid breakdown that is enhanced under stress conditions.

Blée and Joyard (1996) demonstrated that chloroplast envelope membranes synthesize the oxylipins derived from hydroperoxides of polyunsaturated fatty acids. Within a minute, these highly reactive aliphatic molecules are rapidly metabolized into physiologically active lipid-breakdown products: (a) aldehydes and oxoacid fragments, corresponding to the functioning of a hydroperoxide lyase; (b) ketols that were spontaneously formed from allene oxide synthesized by a hydroperoxide dehydratase; (c) hydroxy compounds synthesized enzymatically by a system that has not yet been characterized; and (d) oxoenes resulting from the hydroperoxidase activity of a lipoxygenase. The same metabolism was demonstrated in envelope membranes from nongreen plastids of cauliflower buds (E. Blée and J. Joyard, unpublished results). None of these activities was detected in the stroma or in the thylakoids. Envelope membranes therefore play a central role in the formation of biologically active oxylipins (and especially of the precursor for jasmonate, 12-oxo-phytodienoic acid), and this demonstrates that compartmentation of biosynthesis of lipid-derived molecules is essential for their biological function. The envelope enzymes are important for plant development regulation and to generate signals. Arabidopsis mutants devoid of trienoic fatty acids were unable to produce jasmonate from 18:3 and as a result did not produce any seed and were highly susceptible to pathogens (for review, see Miquel and Browse, 1998).

Plastids are a source of lipid-derived signaling molecules. For example, recent observations of *C. reinhardtii* mutants suggest that some envelope-derived chlorophyll precursors (Mg-protoporphyrin IX or its dimethyl ester but not protoporphyrin IX, protochlorophyllide, or chlorophyllide) can activate the light-signaling pathway controlling the expression of some nuclear genes (Kropat et al., 1997). In addition, some major plant hormones (e.g. ABA and GAs) derive from terpenoid compounds synthesized within chloroplasts (Fig. 3; for review, see Barbier-Brygoo et al., 1997). The involvement of envelope membranes needs to be addressed to understand how compartmentation participates in the regulation of intracellular signaling. ABA is a cleavage product of xanthophylls (e.g. violaxanthin and neoxanthin). The biochemical lesions of the *aba1*, *aba2*, and *aba3* Arabidopsis mutants have been identified (for review, see Koornneef et al., 1998). The gene at the *aba1* locus of Arabidopsis was identified and characterized in *N. plumbaginifolia* to encode zeaxanthin epoxidase (Marin et al., 1996). The *aba2* mutant in Arabidopsis is impaired in the conversion of xanthoxin (made from 9-*cis*-neoxanthin) to ABA-aldehyde. GA biosynthesis is a two-step process

involving first plastids (up to *ent*-kaurene biosynthesis) and then extraplastidial compartments (where GA12-aldehyde is converted into GAs). This suggests that envelope membranes are involved at some stage in GA biosynthesis (for review, see Barbier-Brygoo et al., 1997).

PLASTID ENVELOPES CONTAIN A FAMILY OF PHOSPHATE TRANSLOCATORS

During photosynthesis, triose phosphate and Pi cross the chloroplast inner envelope membrane through the phosphate/triose phosphate translocator (for review, see Flügge et al., 1996; Fig. 4). This protein is the major inner envelope protein (15%–20% of the total envelope proteins), it was the first envelope protein to be purified and functionally reconstituted in liposomes, and its cDNA was the first cloned plant transport system. According to the requirements of different photosynthetic or heterotrophic tissues, the inner envelope membranes from either chloroplasts or nongreen plastids contain different antiport systems that exchange phosphate for triose phosphates, PEP, or hexose phosphates. For example, in nongreen plastids from heterotrophic tissues, hexose phosphates are one of the major forms in which carbohydrates are imported for starch and erythrose-4-phosphate synthesis and for the production of reducing power (via glycolysis and the oxidative pentose phosphate pathway) for nitrite and sulfur assimilation (for review, see Browsher et al., 1996). In addition, ATP is imported into these plastids for various biosynthetic processes (e.g. starch, fatty acid, and amino acid biosynthesis).

In leaves the chloroplast envelope contains a PEP/phosphate transporter that imports PEP for the biosynthesis of aromatic compounds (the shikimic acid pathway). In C4

mesophyll chloroplasts a similar PEP/phosphate transporter is probably involved in the export of PEP for carboxylation to oxaloacetate by cytosolic PEP carboxylase. In leaves the interaction between carbon and nitrogen metabolism involves two dicarboxylate transporters of the inner envelope membrane, a 2-oxoglutarate/malate transporter that imports 2-oxoglutarate into chloroplasts for the Gln synthetase/glutamate synthase system essential for the fixation of NH_3 that derives from nitrite reduction or photorespiration and a glutamate/malate transporter that exports glutamate. Molecular data are now available for several of these transporters in photosynthetic and nongreen tissues. For example, Kammerer et al. (1998) compared the amino acid sequences deduced from cDNAs obtained from a wide diversity of plastids and tissues and encoding three types of carbon transporters (Glc-6-P/phosphate, triose phosphate/phosphate, and PEP/phosphate translocators). These transporters have only slight but highly significant similarities, especially in the hydrophobic membrane-spanning domains.

OTHER TRANSPORTERS REGULATE CHLOROPLAST METABOLIC ACTIVITY

In addition to these transporters, envelope membranes contain a series of ion channels, pumps, permeases, pore proteins, and other substances (Fischer et al., 1994; Mi et al., 1994; Heiber et al., 1995; Pohlmeier et al., 1997) essential for the functional integration of plastids within cells. Most of these proteins have been characterized in chloroplasts; little is known about nongreen plastids. For example, Fischer et al. (1994) found that the outer envelope membrane of nongreen plastids contains a porin highly homologous to that of mitochondria but different from the chloroplast porin that remains to be identified.

Finally, the involvement of envelope membranes in the regulation of photosynthetic metabolism is probably more complex than expected. Rolland et al. (1997) recently disrupted the *ycf10* chloroplast gene (encoding an inner envelope protein) in *C. reinhardtii* and examined the phenotype of the resulting homoplasmic mutants. Mass-spectrometric measurements with either whole cells or isolated chloroplasts revealed that the *ycf10* deficiency affects both CO_2 and HCO_3^- uptake, suggesting the existence of a *ycf10*-dependent system that promotes efficient inorganic carbon uptake into chloroplasts (Fig. 4). One explanation for this observation is that Ycf10 is associated with a system in the chloroplast envelope involved in uptake of both HCO_3^- and CO_2 into the chloroplast. However, another interpretation is that the observed effect on inorganic carbon uptake in the *ycf10*-deficient mutant could only be indirect. Ycf10 might be involved in pH regulation and could be associated with elements of the chloroplast envelope redox chain (Jäger-Vottero et al., 1997) that may be involved in proton extrusion and in the export of photosynthetic reducing power to the cytosol. We do not know which hypothesis is valid, but clearly the passive diffusion of CO_2 across envelope membranes is Ycf10-dependent and is not fast enough to sustain the observed rates of CO_2 fixation by chloroplasts.

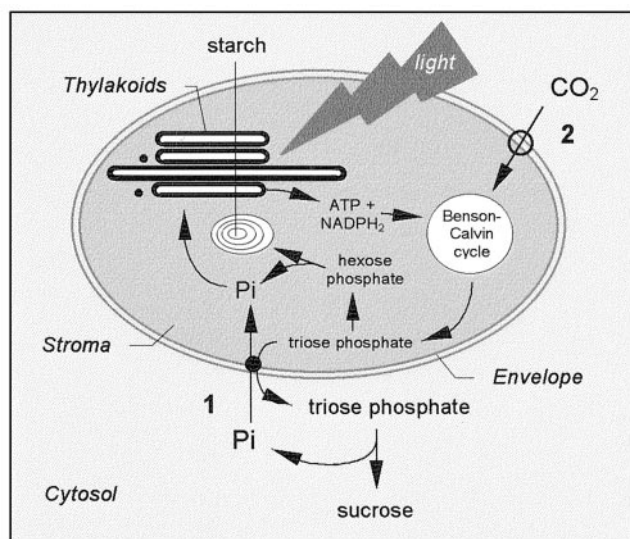


Figure 4. Envelope membranes and the regulation of photosynthesis. In addition to the translocator (1) that exchanges the cytosolic phosphates for triose phosphates that are formed during photosynthesis, the inner envelope membrane contains a protein-dependent system that promotes efficient inorganic carbon uptake into chloroplasts (2). This protein is encoded by a chloroplast gene (*ycf10*).

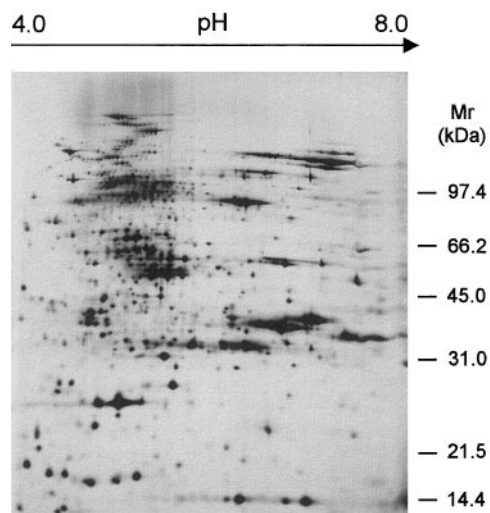


Figure 5. Separation of envelope membrane polypeptides from spinach chloroplasts by two-dimensional electrophoresis. Such a separation demonstrates the complexity of the polypeptide pattern of envelope membranes, despite the fact that it provides only a partial view of the envelope polypeptide pattern, since (a) envelope proteins with pI values greater than 8 are not separated in this system (many envelope transporters have pI values greater than 9) and (b) the most hydrophobic envelope proteins do not enter into these gels. Adapted from Adessi et al. (1997).

The characterization of these proteins in plastid envelope membranes demonstrates the flexibility of plastid metabolism in reflecting that of the various tissues in which they are found.

CONCLUDING REMARKS

The purpose of this short overview is to present the complexity of the plastid envelope biochemical machinery and its importance in cell metabolism, especially as a major site in plant cells for membrane biogenesis. The functional studies of plastid envelope membranes results in the characterization of a continuously increasing number of enzymatic activities. Envelope membranes are the site of transport of metabolites, proteins, and information between plastids and surrounding cellular compartments. They catalyze the biosynthesis of a wide variety of specific plastid constituents that may give rise to signaling molecules derived from these compounds. The complexity of the envelope biochemical machinery is further demonstrated by two-dimensional gel electrophoresis analyses of envelope proteins (Fig. 5). In contrast, only a limited number of envelope proteins have been purified, mostly because of the difficulty in handling such lipid-rich membranes. Furthermore, of the several cDNAs encoding envelope proteins that may have been obtained (for instance analysis of the envelope import machinery continuously generates such cDNAs), only a few of them correspond to proteins with known functions, and most of them do not have homologs in other organisms. There is a major gap between the diversity of envelope polypeptides and the numerous enzymatic activities described above. Further work is

therefore required to characterize envelope proteins and their functions, not only in chloroplasts but in all plastid types. The difficulty involved in the biochemical study of envelope membranes in most tissues (especially nongreen tissues) requires the use of additional genetic and molecular approaches.

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