CORRECTIONS

Journal of Integrative Plant Biology 2007, 49 (8): 1100–1111

1. In line 20 of the right column of page 1102, the estimated number of total proteins in chloroplasts without most outer envelope membrane proteins that do not carry predictable transit peptides in Arabidopsis thaliana is 2200 (2100 + 87), instead of 3000. This number, of course, does not account for those in the chloroplast interior which are synthesized without canonical transit peptides.

2. In Table 1 (page 1103), four homologous GTPases, Toc159, Toc132, Toc120, and Toc90, are mistakenly predicted to be anchored to the membrane via C-terminal α-helical regions. Although the C-terminal portions appear to anchor these proteins to the lipid bilayers, there is no clear predictable hydrophobic domain in these sequences (Bédard and Jarvis 2005). Thus, their structures should be denoted as “X”.

3. atToc64-I is included in Table 1, but the reference #9 (Qbadou et al. 2006) does not discuss its localization in the outer envelope membrane at all. Chew et al. (2004 FEBS Lett 557:109-114) showed that a fusion protein with GFP in its C-terminus was found in nucleus, but not in plastids. Thus, the reference #9 should be deleted from the atToc64-I line, and it should be noted that its outer envelope localization has not been confirmed yet.
The chloroplast outer envelope membrane: The edge of light and excitement

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Abstract

The chloroplast is surrounded by a double-membrane envelope at which proteins, ions, and numerous metabolites including nucleotides, amino acids, fatty acids, and carbohydrates are exchanged between the two aqueous phases, the cytoplasm and the chloroplast stroma. The chloroplast envelope is also the location where the biosynthesis and accumulation of various lipids take place. By contrast to the inner membrane, which contains a number of specific transporters and acts as the permeability barrier, the chloroplast outer membrane has often been considered a passive compartment derived from the phagosomal membrane. However, the presence of galactoglycerolipids and \( \beta \)-barrel membrane proteins support the common origin of the outer membranes of the chloroplast envelope and extant cyanobacteria. Furthermore, recent progress in the field underlines that the chloroplast outer envelope plays important roles not only for translocation of various molecules, but also for regulation of metabolic activities and signaling processes. The chloroplast outer envelope membrane offers various interesting and challenging questions that are relevant to the understanding of organelle biogenesis, plant growth and development, and also membrane biology in general.

Key words: chloroplast envelope; lipid biosynthesis; outer membrane; protein import; putative solute channel.

Received 6 Mar. 2007  Accepted 24 Apr. 2007

Supported by the Department of Pomology of the University of California at Davis (2002–), the Agricultural Experiment Station of the University of California (2002–), National Research Initiative of the USDA-CSREE (2003–2006), the UC Discovery Grant (2003–2007), and the California Citrus Research Board (2007–)

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doi: 10.1111/j.1672-9072.2007.00543.x
The Chloroplast Outer Envelope Membrane Proteome

Figure 1. The chloroplast envelope.

A chloroplast of an *Arabidopsis* mesophyll cell examined under an electron microscope. The part of the chloroplast envelope is enlarged and the outer and inner membranes are indicated. The contact site of the two membranes is circled. Bar, 1 μm. SG and M indicate a starch grain and a mitochondrion, respectively.

membrane has often been considered a passive compartment. It was shown to be permeable to compounds of low molecular weight (Heldt and Sauer 1971; Flügge 2000; Soll et al. 2000). In addition, the chloroplast outer membrane has been described as a remnant of the vacuolar membrane, which was used by the host cell to engulf the symbiont (Whatley and Whatley 1981). These viewpoints may have made the outer envelope a rather non-attractive material for scientific research. However, especially in the last decade, a number of proteins have been identified in the chloroplast outer envelope and their intriguing properties revealed by biochemical, biophysical, and genetic studies. In addition, the similarity between the outer membranes of the chloroplast envelope and those of extant cyanobacteria in lipid and protein compositions strongly supports the common evolutionary origin of the two membranes (Cavalier-Smith 1982; Keegstra et al. 1984; Joyard et al. 1991; see below).

This review aims to stimulate interest in the chloroplast outer envelope. To do so, overviews of its general features, as well as a list of proteins identified or predicted to be in this membrane are provided. For readers who wish to learn more, there are outstanding reviews on chloroplast protein import (Soll and Schleiff 2004; Bédard and Jarvis 2005; Kessler and Schnell 2006; Smith 2006), roles of envelope lipids in protein import (Bruce 1998), lipid metabolism and trafficking (Dörmann and Benning 2002; Awai et al. 2006; Jouhet et al. 2007), and solute transporters in the plastid envelope (Weber et al. 2005). Excellent comprehensive reviews on the chloroplast envelope biology were also published previously (Douce and Joyard 1990; Joyard et al. 1991, 1998; Block et al. 2007).

Unique Features of the Chloroplast Outer Envelope Membrane

Traditionally, leafy tissues such as pea seedlings grown in the greenhouse or spinach purchased at local supermarkets have been used as a source of chloroplasts. Early studies developed procedures for fractionation of the outer envelope membrane from these chloroplasts by density-gradient centrifugations (Cline et al. 1981; Block et al. 1983a) and revealed its unique features in lipid and protein compositions.

First, the weight to weight ratio of lipids to proteins in the chloroplast outer envelope is 2.5–3.0, which is comparable to that of tonoplast (2.3) but is much higher than that of the chloroplast inner envelope (0.8–1.0) (Joyard et al. 1991; Moreau et al. 1998). Thus, lipid composition may be critical for the function of the chloroplast outer membrane. Interestingly, the outer membranes of Gram-negative bacteria consist of about half their mass as proteins (Koebnik et al. 2000), which is similar to the inner but not to the outer membrane of the chloroplast envelope.

Second, the chloroplast envelope and thylakoids as well as cyanobacterial membranes are unique biological membranes in that they are rich in galactoglycerolipids (Wada and Murata 1989; Joyard et al. 1991; Moreau et al. 1998). The chloroplast inner envelope and thylakoids are almost identical in their glycerolipid composition, consisting of monogalactosyl diacylglycerol (MGDG) which tends to form non-bilayer structures, digalactosyl diacylglycerol (DGDG), and phosphatidyl glycerol (PG) in the molar percentage of 55–57, 27–30, and 7–9, respectively. By contrast, the outer envelope contains a significant amount of phosphatidyl choline (PC) (32%) in addition to MGDG (17%), DGDG (29%), and PG (10%). Interestingly, the two polar lipids, PC and PG, are distributed asymmetrically in the outer envelope. PC is mainly located in the cytoplasmic site (outer leaflet), whereas PG is exclusively present in the inner leaflet (Dorne et al. 1985). Recently, PC in the outer leaflet of the chloroplast envelope was shown to be converted to diacylgalactolipids by cytosolic phospholipase D and phosphatidic acid phosphatase (Andersson et al. 2004). Thus, the asymmetrical distribution of PC in the chloroplast outer envelope may be relevant to the lipid metabolism. In addition to these lipids, isoprenoid-derived compounds (i.e. carotenoids (Douce et al. 1973; Markwell et al. 1992) and
prenylquinones such as plastoquinone-9 and tocopherol (Soll et al. 1985)) have been identified in the chloroplast outer envelope. These compounds play important roles in light-harvesting and photoprotection in the internal chloroplast membranes (i.e. thylakoids). The main carotenoid in the envelope membranes violaxanthin is known to be depleted following light stress (Jeffery et al. 1974). Violaxanthin de-epoxidase, which converts violaxanthin to zeaxanthin was found in thylakoids, but not in the envelope, and the exchange of violaxanthin between these two membranes was proposed (Siefermann-Harms et al. 1978). A recent study has demonstrated that MGDG and DGDG have different effects on violaxanthin de-epoxidase activity as well as violaxanthin solubility, which implies that the intra-chloroplastic exchange of violaxanthin may be due to the difference between the envelope and thylakoids in their galactolipid composition (Yamamoto 2006). Furthermore, an interesting possibility was suggested recently that carotenoids in the outer envelope may be converted by a carotenoid cleavage dioxygenase in the cytoplasm to apocarotenoids, which may act as signaling molecules that are important for plant development and survival (Bouvier et al. 2005a). Functions of other isoprenoid compounds in the envelope membranes remain largely unexplored.

Early studies have also revealed two interesting features of the chloroplast outer envelope proteins. The first is their structure: in addition to the proteins anchored to or embedded in the lipid bilayer by one or more \( \alpha \)-helical transmembrane domains, the outer envelope consists of proteins that are predicted to be integrated into the membrane with \( \beta \)-strands that can form a stable pore-like structure called \( \beta \)-barrel. \( \beta \)-Barrel membrane proteins have been found almost exclusively in the outer membranes of Gram-negative bacteria and the endosymbiotic organelles (i.e. chloroplasts and mitochondria) (Wimley 2003). The second unique feature of the chloroplast outer membrane proteins is their targeting mechanism. With a few exceptions, nuclear-encoded proteins found in the interior of chloroplasts are synthesized with an N-terminal extension called a transit peptide, which is necessary and sufficient for targeting the protein to the organelle (Chua and Schmidt 1979; Bruce 2001). The transit peptide-dependent protein targeting is called the general pathway and its mechanism has been studied extensively. In addition, several prediction programs for the transit peptide, such as ChloroP (Emanuelsson et al. 1999), iPSORT (Bannai et al. 2002), PCLR (Schein et al. 2001), and Predotar (http://www.inra.fr/predotar/), have been developed. By contrast to the proteins targeted to the interior of chloroplasts, those found in the outer envelope usually do not carry the transit peptide (Schleiff and Klösgen 2001). Interestingly, available data suggest that some outer envelope proteins may use part of the general pathway for their targeting (for review, see Hofmann and Theg 2005).

In summary, the presence of galactolipids and \( \beta \)-barrel proteins strongly supports the common ancestral origin of outer membranes of chloroplasts and extant cyanobacteria. Phosphatidylcholine and \( \alpha \)-helical transmembrane proteins in the chloroplast outer envelope, which are not detected in the modern cyanobacteria, may have been added during the evolution of the organelle.

**The Chloroplast Outer Envelope Membrane Proteome: How Many Proteins are Required to Make the Outer Envelope?**

Recent completion of genome sequencing (Arabidopsis Genome Initiative 2000), development of genetic tools (e.g. Alonso et al. 2003), and establishment of efficient methods for isolation of intact chloroplasts (e.g. Fitzpatrick and Keegstra 2001) have made Arabidopsis an attractive system alternative to pea or spinach to study biological problems of chloroplasts. Recently, Richly and Leister (2004) evaluated several prediction programs extensively and proposed that about 2100 proteins are synthesized with transit peptides on the Arabidopsis cytoplasmic ribosomes. There are 87 proteins encoded in the Arabidopsis chloroplast genome, none of which appears to be localized to the outer envelope (Sato et al. 1999). These studies estimate about 3000 proteins in chloroplasts, which, however, do not consider most of proteins in the outer envelope that do not contain predictable transit peptides. How many proteins, then, are there in the outer membrane of Arabidopsis chloroplasts? Addressing this question has to depend on individual studies. Examining the recently-developed proteome of the “mixed” envelope from Arabidopsis chloroplasts (Ferro et al. 2003; Froehlich et al. 2003; Friso et al. 2004) is also useful.

Table 1 lists outer membrane proteins of the Arabidopsis chloroplast envelope whose location has been confirmed by various methods or predicted based on their sequence similarity to known proteins. Major proteins identified and characterized well so far are those involved in protein import, solute exchange, and lipid biosynthesis. Addition of other proteins of known and unknown functions makes a total of 34 proteins in the list (Table 1).

**Protein Import Components and their Homologs**

Extensive studies by various laboratories in the last decade or so using chemical cross-linking, co-immunoprecipitation, antibody inhibition, and translocation intermediates resulted in the identification of several components involved in protein import at the envelope membranes of pea chloroplasts. These proteins are designated as Toc and Tic (Translocon at the outer- and the inner-envelope-membranes of chloroplasts) proteins (Schnell et al. 1997). According to the original nomenclature, (1) the designation Toc is followed by a number indicating the molecular
mass of the protein in kilodalton, (2) a two-letter prefix (in lower case) indicates the species of origin of the component, and (3) this is restricted to proteins whose specific functions to envelope translocation are supported by experimental evidence (Schnell et al. 1997). For example, psToc75 is a 75-kDa component of the Toc complex from pea (Pisum sativum) whose function has been established by various studies (see below). Later, when sequences of the Arabidopsis genome became available, an additional designation system which includes the chromosomal locations of the genes was introduced (Jackson-Constan and Keegstra 2001). According to this, atToc75-III is a protein homologous to psToc75 and is encoded in the chromosome III of the Arabidopsis genome. This system is, of course, restricted to proteins from organisms whose genomic information is available, such as Arabidopsis and rice. Currently, designations depend on individual components. This review follows the commonly-used nomenclature and defines components from pea without the two-letter prefix (e.g. Toc75 indicates psToc75).

Among Toc components identified so far, three proteins are considered the core constituents in pea chloroplasts based on their ability to reconstitute precursor protein translocation in artificial liposomes (Schleiff et al. 2003b). They are two homologous Guanosine triphosphatases (GTPases), Toc159 and Toc34, which are anchored to the membrane with α-helices and expose their large N-terminal portions to the cytoplasmic surface, and a β-barrel membrane protein Toc75. The two GTPases are postulated to be involved in recognition and also in the early stage of translocation of the precursor proteins. Mechanistic details by which Toc159 and Toc34 mediate these processes have been a matter of debate (for reviews, see Kessler and Schnell 2004, 2006; Li et al. 2007). Toc75 is one of the most abundant proteins in the chloroplast outer envelope.

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**Table 1.** Proteins identified or predicted to be in the outer membrane of the Arabidopsis chloroplast envelope

<table>
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<th>AGI number</th>
<th>Namea</th>
<th>Sizeb (kb)</th>
<th>Structurec</th>
<th>Proteomed</th>
<th>Function</th>
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<td>1,6</td>
</tr>
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<td>atToc120</td>
<td>120</td>
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<td>Yes</td>
<td>Preprotein recognition/translocation</td>
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<td>atToc90</td>
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<td>VDACa</td>
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<td>Yes</td>
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<td>VDACa</td>
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DGD, digalactosyl diacyl; LAC9, long chain acyl-CoA synthetase 9; MDG, monogalactosyl diacyl; OEP, outer envelope protein; OMP, outer membrane protein; PDV, plastid division; PORA, protocholophyllide oxidoreductase A; THF1, thylakoid formation 1; Toc and Tic, translocon at the outer- and the inner-envelope-membranes of chloroplasts; VDAC, voltage-dependent anion channel.

Proteins from various plastid types and a number of plant species can be targeted properly to chloroplasts isolated from pea seedlings, and at least some of the import components appear to be conserved (Dávila-Aponte et al. 2003). Thus, one might assume that a non-specific general pathway mediates protein import into plastids. This assumption was, however, questioned when genes encoding multiple isoforms for some of Toc components (i.e. four isoforms for Toc159 and two for Toc34) were identified in the Arabidopsis genome (for review, see Jackson-Constan and Keegstra 2001) (Table 1). Analyses of gene expression patterns as well as reverse-genetic and biochemical studies have led to a model that each isoform has a preference for precursor substrates: namely, two Toc159 homologs, atToc159 and atToc90, and a Toc34 homolog, atToc33, may be involved in the import of photosynthetic proteins, whereas another two Toc159 homologs, atToc132 and atToc120, and the second Toc34 homolog, atToc34, may be responsible for the import of proteins with non-photosynthetic functions (Jarvis et al. 1998; Bauer et al. 2000; Kubis et al. 2003, 2004; Ivanova et al. 2004; Smith et al. 2004; Hiltbrunner et al. 2004). The Arabidopsis genome contains four genes that encode apparent homologs to Toc75 in the chromosomes I, III, IV, and V, respectively (Jackson-Constan and Keegstra 2001; Eckart et al. 2002). Among them, atTOC75-I is a pseudo-gene

### Table 1. Continued

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<tr>
<th>AGI number</th>
<th>Namea</th>
<th>Sizeb</th>
<th>Structurec</th>
<th>Proteomed</th>
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<td>C?</td>
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<td>Division</td>
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<td>34</td>
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"Toc12h, fatty acid hydroperoxide lyase (HPLh), and OMP24h are proteins identified in this study, which are apparently homologous to Toc12 from pea, HPL from tomato, and OMP24 from spinach, respectively. The presence of VDAC-like proteins (At3g01280 and At5g15090) in the chloroplast outer membrane may be highly speculative.

"Predicted size in kDa. Two proteins, atToc75-III and THF1, have been shown to contain N-terminal cleavable targeting sequences, and sizes of their mature forms are indicated in the parentheses.

"Proteins predicted to be embedded in the lipid bilayer via a single (N, signal-anchor means locating its N-terminus in the intermembrane space; C, tail-anchor means locating its N-terminus in the cytoplasm), or multiple (P, polytopic) α-helices, β-strands (B) are indicated. Those without any obvious transmembrane domains are indicated as X.

"Proteins identified in the chloroplast envelope proteome (Friso et al. 2004) are indicated as “Yes”.


DGD, digalactosyl diacyl; LAC9, long chain acyl-CoA synthetase 9; MDG, monogalactosyl diacyl; OEP, outer envelope protein; OMP, outer membrane protein; PDV, plastid division; PORA, protocholophyllide oxidoreductase A; THF1, thylakoid formation 1; Toc and Tic, translocon at the outer- and the inner-envelope-membranes of chloroplasts; VDAC, voltage-dependent anion channel.
through the outer envelope. In mitochondria, a 30-kDa hydrates, and other metabolite intermediates and products pass solutes including ions, amino acids, nucleotides, lipids, carbohydrates, and other metabolite intermediates and products pass through the outer envelope. In mitochondria, a 30-kDa \( \beta \)-barrel-forming protein called voltage-dependent anion channel (VDAC) or porin mediates transport of a wide variety of metabolites in the outer membrane (for review, see Benz 1994). Flügge and Benz (1984) showed that the chloroplast outer envelope contained a porin-type pore which was permeable to proteins of up to 10 kD. Fisher et al. (1994b) identified in pea root plastids, but not in chloroplasts, a VDAC-like protein that was predicted to contain 16 \( \beta \)-parallel \( \beta \)-strands. Recently, however, Clausen et al. (2004) demonstrated by immunoblotting and protein targeting assays that VDAC-like proteins were undetectable not only in chloroplasts but also in root plastids. Interestingly, two VDAC-homologs encoded by genes At3g01280 and At5g15090, respectively, have been identified in the Arabidopsis chloroplast envelope proteome (Table 1). Both of them, however, have also been found in the mitochondrial proteome (Brugiere et al. 2004). Thus, the identification of the VDAC homologs in the chloroplast outer envelope could be due to the contamination of the mitochondrial fraction.

If a VDAC-like protein is not present, then, which proteins mediate the exchange of various solutes in the chloroplast outer envelope? This question has been extensively addressed by Jürgen Soll and Richard Wagner’s groups who have been reconstituting several proteins into artificial liposomes and characterizing their properties using electrophysiological measurements. They have identified four proteins of 16, 21, 24, and 37 kDa which were named OEP16 (outer envelope protein 16), OEP21, OEP24, and OEP37, respectively, from the outer membrane of the pea chloroplast envelope. Among them, OEP16 is folded into four-\( \alpha \)-helical transmembrane domains (Linke et al. 2004) and has been suggested to be responsible for the transport of amino acids (Pohlmeier et al. 1997). In Arabidopsis, four proteins apparently homologous to OEP16 were identified (Reinbothe et al. 2004) (Table 1). Among them, three proteins, OEP16.1, 16.2, and 16.4, were localized to chloroplasts, whereas AtOEP16.3 was found only in mitochondria (Philippar et al. 2007). Interestingly, OEP16 was also shown to be involved in a substrate-dependent import of protochlorophyllide oxidoreductase A (PORa) by biochemical and genetic studies (Reinbothe et al. 2004; Pollmann et al. 2007). This model has, however, been challenged by another genetic study although they used the same mutant plants (Philippar et al. 2007).

In contrast to OEP16, all of the other three putative channel proteins are predicted to form \( \beta \)-barrel structures. Interestingly, however, extant cyanobacteria do not seem to contain apparent homologs to any of them (Hsu and Inoue, unpublished obs. 2007). OEP21 orthologs from pea and Arabidopsis have been predicted to traverse the membrane with eight \( \beta \)-strands and were shown to form rectifying adenosine triphosphate (ATP)-regulated anion selective channels in artificial liposomes ( Bölter et al. 1999; Hemmler et al. 2006). OEP24 was predicted to contain seven (Pohlmeier et al. 1998) or twelve (Schleiff et al. 2003a) transmembrane \( \beta \)-strands. It was reconstituted to form a slightly cation-selective high conductance channel, which allowed a flux of a wide range of

**Putative Solute Channel Proteins**

In addition to the nuclear-encoded precursor proteins, various solutes including ions, amino acids, nucleotides, lipids, carbohydrates, and other metabolite intermediates and products pass through the outer envelope. In mitochondria, a 30-kDa \( \beta \)-barrel-forming protein called voltage-dependent anion channel (VDAC)
solutest (Pohlmeyer et al. 1998). Quite remarkably, expression of OEP24 cDNA rescued the respiratory deficiency of a yeast mutant that lacked VDAC (Rohr et al. 1999). This is in contrast to the report showing that expression of a potato mitochondrial VDAC cDNA could not complement another porin-deficient mutant of yeast (Heins et al. 1994). Finally, OEP37, which was originally identified by Schleiff et al. (2003a), was reconstituted into liposomes as a cation-selective channel (Goetze et al. 2006). Interestingly, the OEP37-channel showed an affinity to an inner envelope protein Tic32 in vitro although knockout of the atOEP37 gene did not cause any obvious phenotypic defects including import of Tic32 in Arabidopsis chloroplasts (Goetze et al. 2006). Overall, biophysical studies have helped generate ideas regarding the physiological functions of these putative channel proteins.

Proteins Involved in Lipid Metabolism

The chloroplast outer envelope contains various lipids, which are represented by galactolipids, carotenoids, and prenylquinones. A number of enzymes that are responsible for their biosynthesis have been identified (for reviews, see Dörmann and Benning 2002; Bouvier et al. 2005b; DellaPenna and Pogson 2006), but most of them appear to be located in compartments other than the outer envelope. Nonetheless, limited numbers of lipid biosynthetic enzymes have been identified in the Arabidopsis chloroplast outer envelope including four galactolipid galactosyltransferases, DGD1, DGD2, MGD2, and MGD3. The DGD and MGD genes were originally identified by genetic screening of mutant plants that were deficient in DGDG and MGDG, respectively (Dörmann et al. 1995; Jarvis et al. 2000). DGD1 and MGD1 provide the bulk of galactolipids in green tissues, whereas DGD2 and MGD2/3 appear to be responsible for the biosynthesis of galactolipids in non-green plastids and in extraplastic membranes on phosphate starvation (for review, see Benning and Ohta 2005). Both DGD1 and DGD2 were localized in the chloroplast outer envelope (Froehlich et al. 2001a; Kelly et al. 2003). MGD1 was found in the chloroplast inner envelope, whereas MGD2 and MGD3 were suggested to be located in the outer envelope (Awai et al. 2001). Thus, the chloroplast outer envelope clearly plays important roles in the biosynthesis of galactoglycerolipids.

Some fatty acids synthesized in chloroplasts are postulated to be exported to the cytoplasm where they are used as precursors to glycerolipids at the endoplasmic reticulum. This process requires reactivation of the fatty acids by acyl-CoA synthetase in the chloroplast outer membrane (Andrews and Keegstra 1983; Block et al. 1983b). Recently, long chain acyl-CoA synthetase 9 (LACS9) was localized to the chloroplast envelope by in vitro import assay and its in vivo targeting as a green fluorescent protein (GFP)-fusion, and was suggested to be responsible for this fatty acid reactivation (Schnurr et al. 2002). However, its localization in the outer envelope has not been proven yet (Koo et al. 2004).

Finally, two cytochrome P450s have been identified in the chloroplast outer envelope membranes. One of them, fatty acid hydroperoxide lyase, is involved in oxylipin biosynthesis in tomato (Froehlich et al. 2001b), and its apparent Arabidopsis ortholog Ath415440 has been identified in the chloroplast outer envelope proteome (Table 1). The physiological role of another membrane cytochrome P450 CYP86B1 remains unknown (Watson et al. 2001).

Other Proteins

In addition to the proteins described above, various proteins of known and unknown functions have been identified in the chloroplast outer envelope (Table 1). Among them, PDV1 (plastid division 1) and PDV2 were found to be responsible for the recruitment of a cytoplasmic dynamin-related protein ARC5 (accumulation and replication of chloroplasts 5) during plastid division (Miyagishima et al. 2006). A hexokinase, which may be involved in the energization of glucose export, was found in the outer envelope of spinach chloroplasts (Wiese et al. 1999). Recently, however, this protein was demonstrated to be located in mitochondria (Damari-Weissler et al. 2007).

Thylakoid formation 1 (THF1) is the only known protein which is present both in the stroma and outer envelope of chloroplasts, and also one of few outer envelope proteins that are synthesized as a larger precursor with a transit peptide in the cytoplasm (Wang et al. 2004; Huang et al. 2006). The stroma-located THF1 appears to be important for thylakoid formation (Wang et al. 2004; Keren et al. 2005), whereas the outer-envelope-located THF1 may interact in the stromule with a plasma membrane G-protein GPA1, which plays roles in the sugar signaling pathway (Huang et al. 2006).

Outer envelope protein 14 (OEP14) from pea, which is actually a 7-kDa protein but migrates with an apparent molecular mass of 14 kDa on sodium dodecyl sulfate-polyacrylamide gel electrophoresis, is anchored to the membrane via a single α-helical domain in its N-terminus and is facing its soluble domain to the cytoplasm. A 6.7-kDa protein orthologous to OEP14 is one of predominant proteins in the outer membrane of the spinach chloroplast envelope (Koike et al. 1998). Although their functions remain unknown, OEP14 orthologs from pea, spinach, and Arabidopsis have been used as a model to study protein targeting and insertion to the chloroplast outer membrane (Salomon et al. 1990; Li et al. 1991; Li and Chen 1996; Tu and Li 2000; Schleiff et al. 2001; Lee et al. 2001; Tu et al. 2004).

Another protein of unknown function, OMP24 (outer membrane protein 24), which is distinct from OEP24, was identified as one of the major outer membrane proteins of spinach chloroplasts (Joyard et al. 1983). It is unique in its high content of proline and acidic residues (Fischer et al. 1994a). Its putative
homolog At3g52230 has been identified in the *Arabidopsis* chloroplast envelope proteome (Table 1).

### Conclusions and Future Perspectives

Recognition and translocation of nuclear-encoded precursor proteins, the exchange of various ions and metabolites between the cytoplasm and the organelle, and lipid biosynthesis have been the major focuses of the research on the chloroplast outer envelope. Recent multidisciplinary studies have identified proteinaceous components responsible for these processes. In addition, proteins involved in division, sugar-signaling, and those with unknown functions have been found in the chloroplast outer envelope. Because of the lack of predictable transit peptides, identification of chloroplast outer membrane proteins requires vigorous biochemical experiments and also can be assisted by proteomic studies. Table 1 lists 34 proteins (including two VDAC homologs, whose chloroplast localization may be questionable), which should cover the main components of the *Arabidopsis* chloroplast outer envelope. This list underlines the importance of the chloroplast outer envelope not only for translocation of a variety of molecules but also as a critical cellular compartment to regulate various metabolic activities and signaling processes.

The list also provides us with a number of intriguing questions. An obvious first question concerns the physiological roles of the proteins of unknown functions including the putative solute channels. The second question is how these proteins are targeted to and assembled in the chloroplast outer envelope. It has been known that distinct but partly shared pathways are present for the targeting of signal-anchored, tail-anchored, polytopic, and β-barrel membrane proteins to the mitochondrial outer membrane (Rapaport 2003, 2005; Neupert and Herrmann 2007). By contrast, our understanding of protein targeting to the chloroplast outer membrane is limited virtually only to a β-barrel protein with the unusual bipartite transit peptide Toc75 and a signal-anchored protein OEP14 and its orthologs. More work is definitely required to address the question of how unique the protein targeting to the chloroplast outer membrane is. Finally, evolutionary origins of the protein import machineries, especially that of Toc75, have been discussed but not completely understood yet (Reumann et al. 2005; Schleiff and Soll 2005). Addressing these questions requires multidisciplinary approaches and should contribute to advancing our understanding of organelle biogenesis, plant growth and development, and membrane biology in general.

### References


Schnurr JA, Shockey JM, de Boer GJ, Browse JA (2002). Fatty acid export from the chloroplast. Molecular characterization of a
The Chloroplast Outer Envelope Membrane Proteome


