The Phosphatidic Acid Binding Site of the Arabidopsis Trigalactosyldiacylglycerol 4 (TGD4) Protein Required for Lipid Import into Chloroplasts

Zhen Wang, Nicholas Scott Anderson, and Christoph Benning

From the Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, Michigan 48824

Background: The phosphatidic acid (PtdOH) binding site of TGD4 is not yet known.

Results: Amino acids 1–80 and 110–145 of TGD4 represent two PtdOH interacting sequences and TGD4 forms a homodimer in vitro and in vivo.

Conclusion: TGD4 N terminus binds PtdOH while its C terminus interacts with a second TGD4 protein.

Significance: This work reveals the structural functional relationship of an essential chloroplast β-barrel lipid transfer protein.

Chloroplast membrane lipid synthesis relies on the import of glycolipid precursors from the ER. The TGD (TriGalactosylDiacylglycerol) proteins are required for this lipid transfer process. The TGD1, -2, and -3 proteins form a putative ABC (ATP-binding cassette) transporter transporting ER-derived lipids through the inner envelope membrane of the chloroplast, while TGD4 binds phosphatidic acid (PtdOH) and resides in the outer chloroplast envelope. We identified two sequences in TGD4, amino acids 1–80 and 110–145, which are necessary and sufficient for PtdOH binding. Deletion of both sequences abolished PtdOH binding activity. We also found that TGD4 from 18:3 plants bound specifically and with increased affinity PtdOH. TGD4 did not interact with other proteins and formed a homodimer both in vitro and in vivo. Our results suggest that TGD4 is an integral dimeric β-barrel lipid transfer protein that binds PtdOH with its N terminus and contains dimerization domains at its C terminus.

In land plants, glycolipids, such as monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG), and sulfoquinovosyldiacylglycerol (SQDG) comprise ~64% of total membrane lipids (1). Glycolipids are synthesized through two independent pathways: the prokaryotic and the eukaryotic pathways (2). Plastid specific acyltransferases of the prokaryotic pathway are associated with the inner plastid envelope membrane and transfer the acyl groups, which are de novo synthesized in the chloroplast and attached to acyl carrier proteins (ACPs), to glycerol-3-phosphate producing PtdOH. This PtdOH is then dephosphorylated to diacylglycerol (DAG), the direct substrate for glycolipid synthesis. In the eukaryotic pathway, endoplasmic reticulum (ER)-associated acyltransferases use acyl-CoAs derived from plastid-exported acyl groups and glycerol-3-phosphate as substrates, providing PtdOH for the synthesis of extra-plastidic phospholipids, such as phosphatidylcholine (PtdCho) and phosphatidylethanolamine (PtdEtn). A certain portion of lipid precursors returns to the chloroplast, where ER-assembled DAG moieties are incorporated into glycolipids (3). Glycolipids originating from the prokaryotic pathway have a 16-carbon acyl chain in the sn-2 position of the glyceryl backbone, while glycolipids derived from the eukaryotic pathway have an acyl chain of 18 carbons in the same position. This difference has been attributed to the different substrate preferences of acyltransferases in the chloroplast and the ER (4, 5). Plants that utilize both pathways, such as Arabidopsis thaliana, have 16:3 fatty acids in the sn-2 position of their thylakoid lipids, and thus are referred to as 16:3 plants. Other plants, such as maize or castor bean, rely exclusively on the eukaryotic pathway and have mostly 18:3 fatty acids in the sn-2 position of their thylakoid lipids; these plants are designated 18:3 plants (6).

Four Arabidopsis proteins, TGD1–4, are currently known to be involved in the ER-to-chloroplast lipid trafficking process (7–10). Plants with mutations in any of the TGD genes are impaired in the eukaryotic pathway and accumulate an additional lipid, trigalactosyldiacylglycerol (TGDG). The TGD1, 2, and 3 proteins are proposed to form a bacterial-type ABC transporter complex (11), in which TGD1 is a membrane permease, TGD2 a substrate-binding protein, which specifically binds to PtdOH, and TGD3 an ATPase. TGD4 is a predicted β-barrel membrane protein (12, 13) embedded in the chloroplast outer envelope and, like TGD2, specifically binds PtdOH (14). Based on the current findings, it seems likely that the TGD proteins form a lipid transfer conduit to import PtdOH from the ER through the plastid envelopes. Here we describe the identification of two PtdOH binding sequences of TGD4, the lipid bind-

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1 Present address: Dept. of Biological Sciences, Dartmouth College, Hanover, NH, 03755.
2 To whom correspondence should be addressed: Department of Biochemistry and Molecular Biology, Michigan State University, 3280 Molecular Plant Science, East Lansing, MI 48824. Tel.: 517-353-1609; Fax: 517-353-9334; E-mail: benning@msu.edu.

3 The abbreviations used are: DAG, diacylglycerol; Col-2, Columbia-2; ER, endoplasmic reticulum; TGDG, trigalactosyldiacylglycerol; PtdOH, phosphatidic acid; PtdCho, phosphatidylcholine.
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...ing properties of TGD4 proteins in 18:3 plants, and the composition of the TGD4 complex.

EXPERIMENTAL PROCEDURES

Expression and Purification of DsRED-TGD4 Proteins—The truncation mutants T1-T21 were generated starting with construct pLW01/DsRED-TGD4 as the template for polymerase chain reaction (PCR) (14). The PCR products were subsequently inserted into the pLW01 vector using SacI and NotI restriction sites. M1-M9 point mutations were generated by site-directed mutagenesis (Stratagene, La Jolla, CA) using pLW01/DsRED-TGD4 T14 (AA 110–145) as the template and M10-M11 were commercially synthesized (IDT, San Jose, CA). To isolate TGD4 homologs from Ricinus communis (RcTGD4) and Zea mays (ZmTGD4), cDNAs obtained by reverse transcription from isolated total RNA were used as the template for PCR. RcTGD4 and ZmTGD4 were inserted into the BamHI/NotI and EcoRI/NotI restriction sites of the pLW01/DsRED-His vector, respectively. Primer sequences used in this study are summarized in supplemental Table S1. Sequencing was performed by the Research Technology Support Facility (RTSF) at Michigan State University to verify the accuracy of cloned fragments. Recombinant proteins were produced in Escherichia coli strain BL21 (DE3) and purified as previously described (14). Briefly, all proteins were produced at 16 °C overnight after adding 1 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) to the cell culture at an A600 of 0.6 except for T1, T3, T17-T20, M10, M11, which were produced by incubation at 28 °C for 3 h. Cells were resuspended in lysis buffer (50 mM Na2HPO4, 300 mM NaCl, 10 mM imidazole, protease inhibitor mixture (Roche, Indianapolis, IN), 1% forskolin-12 (Affymetrix, Santa Clara, CA), pH 8.0) and lysed by incubating with lysozyme (Sigma) at a final concentration of 0.2 mg/ml for 30 min followed by sonication using the sonicator 3000 with a microprobe (Misonix, Farmingdale, NY) for 2 min with 10 s pulses. Recombinant proteins were purified to homogeneity by Ni2+-NTA-agarose (Qiagen, Valencia, CA) gradient by centrifugation at 4,000 g. Purified proteins were dialyzed with Tris-buffered saline (TBS: 10 mM Tris-HCl, pH 7.0, 0.1 mM NaCl) with 2 mM choline chloride using Amicon centrifugal filter devices with 10 kDa cutoff (Millipore, Billerica, MA). Purified proteins were quantified by RC DC protein assay (Bio-Rad, Hercules, CA).

Liposome Association Assay and Data Quantification—Multi-lamellar liposomes composed of dioleoyl-PtdCho and dioleoyl-PtdOH with different w/w ratios as mentioned in the figure legends were prepared as previously described (14). Briefly, 250 μg of total lipids (Avanti, Alabaster, AL; Larodan, Sweden) dried under a stream of nitrogen were hydrated by adding 500 μl of TBS with 0.2 mM choline chloride and incubating at 37 °C for 1 h. Multi-lamellar liposomes were obtained by vortexing for 2 min at the highest speed. Purified proteins (0.25 μM) were incubated with liposomes on ice for 30 min. Unbound proteins were separated from liposome-bound proteins by centrifugation at 13,000 × g for 10 min at 4 °C followed by two washes with TBS containing 0.2 mM choline chloride. The liposome-protein pellet was examined on a SDS-PAGE gel (15) followed by Coomassie Brilliant Blue R-250 (Sigma) staining. Quantitative analysis was based on densitometry using ImageJ software (16) with three technical repeats. The amount of liposome-bound full-length DsRED-TGD4 was set at 100% and the final data were normalized to individual loading controls. The results in Fig. 6C were fitted to Hill’s equation (Equation 1) using Origin software (OriginLab, Northampton, MA),

\[
\frac{n}{L^n + K_d}
\]  
(Eq. 1)

where “n” is Hill’s number, “L” is ligand concentration, and Kd is the dissociation constant.

Protein Lipid Overlay Assay—Lipid strips were prepared as previously described (14). Briefly, 1 nmol of lipids in 1 μl of buffer (25% v/v chloroform, 50% v/v methanol, 10 mM HCl, 1% Ponseau S) were spotted onto a nitrocellulose membrane (GE Healthcare, Piscataway, NJ) and dried in a fume hood for 1 h. Finished membranes were blocked in TBST (TBS with 0.1% Tween-20) buffer plus 3% Bovine Serum Albumin (BSA) for 2 h at room temperature. Purified proteins were added to the blocking solution at 1 μg/ml final concentration and incubated with lipid strips at 4 °C overnight followed by washing in TBST buffer 3 × 10 min and immunoblotting with anti-His (C-term) antibody (Invitrogen, Grand Island, NY) at a dilution of 1:5000. Secondary antibody anti-mouse horseradish peroxidase (Bio-Rad) (dilution: 1:20,000) was added for 0.5 h followed by washing in TBST buffer 6 × 10 min. Signal was detected using a chemiluminescence kit from Sigma.

Plant Growth Conditions—Arabidopsis thaliana ecotype Col2 transformed with N-terminal HA-tag labeled TGD4 (14) were grown on agar-solidified Murashige-Skoog (MS) medium supplemented with 1% sucrose for 3 weeks as previously described (17, 18). Aerial parts were harvested for chloroplast isolation.

Chloroplast Preparation—Arabidopsis chloroplasts were prepared as previously described with modifications (19). Briefly, 4-week-old seedlings harvested from MS solid medium were ground in grinding buffer (330 mM sorbitol, 50 mM HEPES-KOH, pH 8.0, 2 mM EGTA, 0.1% w/v BSA with 5 mM MgCl2) and separated on a 40 and 80% discontinuous Percoll (Sigma) gradient by centrifugation at 4,000 rpm for 10 min. The intact chloroplasts collecting at the interface were isolated and washed once with buffer (330 mM Sorbitol, 50 mM HEPES-KOH, pH 8.0, 5 mM MgCl2) followed by centrifugation at 700 × g for 5 min.

Blue-Native Electrophoresis and Immunoblots—100 μg of isolated chloroplasts from HA-TGD4 transgenic Arabidopsis or 20 μg of purified DsRED-TGD4 recombinant proteins dissolved in Ni-NTA elution buffer with 0.1% foscholine-12, were suspended in a buffer containing 50 mM Bis-tris/HCl (pH 7.0), 500 mM 6-aminocaproic acid, 10% (v/v) glycerol, 1% (w/v) n-dodecyl-β-D-maltoside (Thermo Fisher, Rockford, IL) and protease inhibitor mixture. Debris was removed by ultracentrifugation at 100,000 × g for 10 min. Blue-Native electrophoresis was performed as previously described (20). Proteins on the Blue-Native gel were denatured in a buffer containing 3.3% (w/v) sodium dodecyl sulfate (SDS), 1 M Tris-HCl (pH 6.8) and 4% (w/v) β-mercaptoethanol at 85 °C for 30 min. Denatured proteins were then transferred to a polyvinylidene fluoride (PVDF)
immunoblot membrane (Bio-Rad) followed by blocking with 5% fat-free milk in TBST buffer. Anti-HA antibody 1: 5,000 dilution (Sigma) was used to identify HA-TGD4 protein. The blot was then incubated with anti-mouse horseradish peroxidase (1:20,000 dilution) for 0.5 h followed by six washes in TBST buffer 10 min each. The signal was developed using a chemiluminescence kit.

**Accession Number**—The sequence of *Arabidopsis thaliana* TGD4 can be found in The Arabidopsis Information Resource under the name At3g06960. The sequence of *Ricinus communis* and *Zea mays* TGD4 can be found in GenBankTM under the accession numbers XM_002519286.1 and XM_002519286.1, respectively.

**RESULTS**

**Amino acids 110–145 Are Necessary and Sufficient for PtdOH Binding by TGD4**—To locate the PtdOH binding site(s) within the TGD4 primary sequence, partially overlapping truncation mutants T1-T5 were constructed (Fig. 1A). The N termini of the respective truncations were fused to DsRed to enhance solubility, and the resulting recombinant proteins were produced in *E. coli* and purified to homogeneity using a C-terminal 6× His tag. The proteins were then tested for PtdOH binding in a liposome association assay, wherein proteins interacting with the PtdOH-containing liposome precipitated after centrifugation at 13,000 × g. Protein content of the pellet was analyzed by SDS-PAGE and stained by Coomassie Brilliant Blue. Numbers indicate molecular masses in kDa. T, total protein used in this assay. C, images were quantified by densitometry. Data were normalized with bound DsRed-TGD4 as 100%. Averages and standard deviations were calculated from three repeats.

**FIGURE 1.** Coarse mapping of the TGD4 PtdOH binding domain. A, schematic representation of TGD4 truncation mutants T1-T5 fused with DsRed on the N terminus. White bar, DsRed; black bar, TGD4 fragment; gray bar, 6× His tag; numbers below indicate amino acid positions in the full-length, wild-type sequence. B, multi-lamellar liposomes consisting of 40% (w/w) PtdOH and 60% (w/w) PtdCho were incubated with purified proteins. Proteins bound to the liposome co-precipitated after centrifugation at 13,000 × g. Protein content of the pellet was analyzed by SDS-PAGE and stained by Coomassie Brilliant Blue. Numbers indicate molecular masses in kDa. T, total protein used in this assay. C, images were quantified by densitometry. Data were normalized with bound DsRed-TGD4 as 100%. Averages and standard deviations were calculated from three repeats.

**FIGURE 2.** Fine-mapping the TGD4 PtdOH binding domain. A, schematic representation of N-terminal DsRed fused TGD4 truncation mutants T7-T12. White bar, DsRed; black bar, TGD4 fragment; gray bar, 6× His tag; numbers below indicate amino acid positions. B, same liposome binding assay was performed for truncation mutants T7-T12 as in described in the legend of Fig. 1B. Numbers indicate molecular masses in kDa. T, total protein used in this assay. Images were quantified and data were normalized with liposome-bound T2 protein as 100%. Averages and standard deviations were calculated from 3 repeats. C, T14 (AA 100–150) secondary structure predicted by PROF (PredictProtein). Y-axis represents the likelihood of each structural signature. Note that amino acids 110–145 formed a putative soluble loop.
The protein content of the liposome pellet was then examined by SDS-PAGE, and the protein signals were quantified by densitometric scanning. As shown in Fig. 1, B and C, the N-terminal 1–300 amino acids accounted for the PtdOH binding activity of TGD4, while the C-terminal portion of the protein showed greatly reduced PtdOH binding.

To precisely map the minimum PtdOH binding sequence of TGD4, additional truncation mutants T7 to T12 spanning amino acids 100 to 300 were tested (Fig. 2 A) for their PtdOH binding (Fig. 2 B). Except for T12 (AA 100–125), all truncations up to T11 (AA 100–150) were able to bind PtdOH seemingly as strongly as T2, indicating that amino acids 100–150 are sufficient and amino acids 125–150 are necessary for PtdOH binding. Prediction of the secondary structure suggested that amino acids 100–150 contain an intact soluble loop domain (AA 110–145) (21). Since many of the identified PtdOH binding sites, such as those in mTOR (mammalian Target Of Rapamycin), Opi1p (OverProduction of Inositol), and TGD2, adopt a loop conformation (22–24), we hypothesized that this soluble loop (AA 110–145) represents the minimum PtdOH binding sequence of TGD4.

Additional mutants were constructed to test this hypothesis: T13 (AA 125–145), T14 (AA 110–145), and T15, which is a truncated version of TGD4 that contains the entire TGD4 protein sequence, except that the loop was substituted with a 6× His linker sequence (Fig. 3 A). While T13 only retained partial PtdOH binding activity, T14 still showed full PtdOH binding (Fig. 3 B). To test if these truncations were still specific to PtdOH, a lipid overlay assay was used. The purified proteins were incubated with a hydrophobic membrane onto which different plant lipids were spotted. The proteins were then detected with an anti-His antibody (Fig. 3 C). Both T14 and full-length TGD4 bound exclusively to PtdOH, and with similar strength. T15 bound specifically to PtdOH as well, but the signal was attenuated suggesting weakened PtdOH binding. In the liposome association assay, T15 lost about 50% of the total PtdOH binding activity, suggesting the existence of an additional PtdOH binding sequence (Fig. 3 D). As positively charged amino acids are usually essential for PtdOH binding through electrostatic interactions with the negatively charged PtdOH below indicate amino acids. B, same liposome binding assay was performed for truncation mutants T13-T15 as in Fig. 18. Numbers indicate molecular masses in kDa. T, total protein. Images were similarly quantified as in Fig. 2B and normalized with liposome-bound T11 as 100%. C, membranes spotted with various lipids were incubated with purified proteins. Proteins bound to lipids were detected using anti-His antibody. DAG, dioleoyl-diacylglycerol; DGDG, digalactosyldiacylglycerol; MGDG, monogalactosyldiacylglycerol; PtdCho, dioleoyl-phosphatidylcholine; PtdEtn, dioleoyl-phosphatidylethanolamine; PtdGly, phosphatidylglycerol; PtdIns, dioleoyl-phosphatidylinositol; PtdOH, dioleoyl-phosphatidic acid; PtdSer, dioleoyl-phosphatidylserine; SQDG, sulfoquinovosyldiacylglycerol; TAG, trioleoyl-triacylglycerol. D, same liposome binding assay as in B, except that the PtdOH concentration was 30% (w/w). Data were normalized with liposome-bound DrsRED-ARTGD4 as 100%. Numbers indicate molecular masses in kDa. T, total protein. E, alignment of TGD4 amino acids 110–145 within three species. Black boxes, identical amino acids; gray boxes, similar amino acids; M1–M9, T14 point mutants. T, total proteins. Numbers indicate molecular masses in kDa. T, total protein. F, liposome binding assays with 30% (w/w) PtdOH were conducted with different point mutants. T, total proteins. Numbers indicate molecular masses in kDa. The data were quantified by densitometry with three repeats and normalized with liposome-bound T14 as 100%.
head group (25), we hypothesized that mutating those positive residues in the minimal PtdOH binding domain of TGD4 would affect PtdOH binding. Thus, we changed each positively charged amino acids between residues 110 and 145 to alanine (Fig. 3E) and tested the point mutants' PtdOH binding activity except for M6 (AA 110–145, K140A), which we were unable to produce in E. coli. We also included a point mutant targeting a conserved serine, M1 (AA 110–145, S128A). While no single point mutant abolished PtdOH binding, mutants M1-to-M4 retained only about 60% of PtdOH binding activity compared with T14 (AA 110–145) (Fig. 3F).

A Second PtdOH Binding Domain Was Present in the First 80 Amino Acids of TGD4—As shown in Fig. 1C, T5 (AA 1–100) retained wild-type PtdOH binding activity despite lacking the aforementioned, PtdOH-binding sequence (AA 110–145). We investigated which sequence in this most N-terminal portion of the protein represented by T5 (AA 1–100) is responsible for PtdOH binding. Additional truncation mutants T17–T19 were constructed (Fig. 4A) and their PtdOH binding activity was tested by liposome association assay. Only T19 (AA 1–80) bound to PtdOH while T18 (AA 1–50) and T17 (AA 1–25) did not, indicating that amino acids 50–80 were necessary for PtdOH binding by this fragment (Fig. 4B). This region was also predicted to have a loop structure and was highly conserved among different species (Fig. 4, A and E). Additional mutants were tested, which included a truncation spanning residues 50–80 (T20) and two point mutants, M10 and M11, in the 50–80 AA region that had an alanine in place of a positively charged arginine (Fig. 4A). In contrast to T14 (AA 110–145), T20 (AA 50–80) was not sufficient for PtdOH binding. The truncation mutant T21 that lacked both PtdOH binding sequences (AA 110–145 and AA 1–80) abolished the PtdOH binding activity, indicating that both sequences are necessary in order for TGD4 to bind PtdOH and that these two sequences are the only PtdOH binding sequences in the TGD4 protein.

TGD4 Orthologs of 18:3 Plants Bind PtdOH with High Affinity—Unlike 16:3 plants such as Arabidopsis, which have two pathways for chloroplast lipid synthesis, 18:3 plants depend solely on the eukaryotic pathway. However, the activity of plastidic PtdOH phosphatase was shown to be very low in the 18:3
To study the TGD4 complex in its native environment, and to employ an approach independent of DsRED, which represents a relatively large tag that might affect oligomerization of the fusion protein even though an engineered monomeric version of DsRED was used, chloroplasts from HA-TGD4 transgenic plants were isolated and their protein content was analyzed by Blue-Native PAGE. The TGD4 complex was specifically detected by immunoblotting using an HA antibody. The size of the complex fell between protein markers of 66 and 132 kDa under both resting and lipid-importing conditions (Fig. 6D). Under resting condition, isolated chloroplasts were incubated in an iso-osmotic buffer while in the lipid-importing condition liposomes composed of PtdOH and PtdCho were added to the chloroplast to allow lipid import as was previously shown (17). Both conditions were chosen to account for the possibility that TGD4 may exist in different complexes depending on the conditions. To better estimate the size of the complex, protein marker sizes were plotted against their migration distances. The approximate size of the complex was 115 kDa, which is approximately twice the size of the TGD4 monomer of 53 kDa. Since no further interacting partners were identified by mass spectrometry, and TGD4 did not interact with other known TGD proteins by co-immunoprecipitation, we concluded that TGD4 forms homodimers in vivo.

**DISCUSSION**

TGD4 was identified as a protein involved in ER-to-chloroplast lipid trafficking in a genetic screen (9). However, its molecular function was unknown until recently. TGD4 is a PtdOH binding protein that forms a β-barrel and is embedded in the chloroplast outer envelope membrane (14). Here we provide a detailed molecular and biochemical analysis of TGD4, in particular its PtdOH binding activity. Among the best characterized PtdOH binding proteins in plants are ABI1 (abscisic acid insensitive-1), AtPDK1 (3’-phosphoinositide-dependent kinase-1), PEPC (phosphoenolpyruvate carboxylase), ACBP1

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**Note:** The text is formatted as a natural language reading, focusing on the key points and avoiding excessive technical jargon. It aims to provide a clear understanding of the research findings. The original document contains detailed figures, diagrams, and tables, which are not transcribed here but are integral to the full understanding of the research. The emphasis is on the methodologies, results, and conclusions drawn from the study. The narrative follows a logical flow, ensuring that the reader can follow the progression of the research questions and findings. Key terms and methodologies are defined where necessary, and the context is provided to understand the significance of the research contributions. This approach ensures that the text is both informative and accessible, providing a comprehensive overview of the study.
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Figure 6. TGD4 formed oligomers in vitro and dimers in vivo. A, schematic representation of DsRED-TGD4 N-terminal and C-terminal truncation mutants. White bar, DsRED; black bar, TGD4 fragment; gray bar, 6×His tag; numbers below indicate amino acids. B, Blue-Native PAGE showed that DsRED-TGD4 proteins oligomerized in vitro. The calculated monomer size of each protein was: DsRED, 26.5 kDa; DsRED-TGD4, 80 kDa; DsRED-TGD4N, 59 kDa; DsRED-TGD4C, 46 kDa. Single asterisk: dimer; double asterisk: pentamer. C, DsRED-TGD4 bound to PtdOH in a potentially cooperative manner. Multilamellar liposomes made from 250 μg of total lipid containing 0–70% (w/w) PtdOH were incubated with purified proteins. Liposome-bound proteins were separated by SDS-PAGE and stained with Coomassie Brilliant Blue. Data were normalized to total DsRED-TGD4 as 100% and quantified by densitometry with three repeats and fitted with Hill’s equation with a derived Hill’s number equal to 3. Black dots, DsRED-TGD4; gray squares, DsRED; T, total protein. D, TGD4 forms dimers in vivo. Chloroplast proteins from HA-TGD4 transgenic Arabidopsis in the tgd4−1 background were separated on a Blue-native gel and detected by anti-HA antibody. The monomer size of HA-TGD4 is 53 kDa. 1) HA-TGD4 chloroplast; 2) HA-TGD4 chloroplast incubated with PtdOH/PtdCho (50/50, w/w) liposomes. Vertical numbers: molecular masses in kDa. Migration distance was plotted against molecular mass and fitted with the logarithmic curve. The dashed line indicates the position of the HA-TGD4 band.
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A

B

FIGURE 7. A, functional domains of the TGD4 protein. Amino acids 1–80 and 110–145 encompass PtdOH binding activity and C-terminal amino acids 309–479 are responsible for dimerization. B, diagram of protein complexes involved in the ER-to-chloroplast lipid trafficking pathway. TGD4 embedded in the outer envelope of the chloroplast (OE) forms a β-barrel dimer complex while the TGD1, -2, -3 complex forms an ABC transporter in the inner envelope of the chloroplast (IE). Together these two complexes are proposed to import PtdOH from the ER to the inside of the chloroplast for the synthesis of thylakoid lipids.

several sequences (Fig. 7) and presumably transfers it across the outer envelope. The TGD2 protein of the TGD1, -2, -3 transporter complex extracts PtdOH from the inner leaflet of the outer envelope and presents it to the TGD1 permease, which transports PtdOH across the inner envelope at the expense of ATP hydrolyzed by TGD3. This hypothesis predicts that PtdOH has to be converted to DAG on the stroma side of the inner envelope membrane as DAG is the direct substrate for the bulk synthesis of chloroplast glycolipids. Further corroboration of this hypothesis would be aided by crystal structures of lipid substrate complexes, reconstitution of functional lipid transfer complexes in vitro, and a better understanding of location and substrate specificity of the PtdOH phosphatases predicted to be involved in the process.

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