Isolation and characterization of a phosphatidylglycerophosphate phosphatase1, PGPP1, in
Chlamydomonas reinhardtii

Chun-Hsien Hung^a, Koichi Kobayashi^b, Hajime Wada^{b,c} and Yuki Nakamura^{a,d*}

^aInstitute of Plant and Microbial Biology, Academia Sinica, Taipei, Taiwan, ^bDepartment of Life Sciences, Graduate School of Arts and Sciences, The University of Tokyo, Japan, ^cCREST, Japan Science and Technology Agency, Saitama, Japan, ^dPRESTO, Japan Science and Technology Agency, Saitama, Japan

*Corresponding author: Yuki Nakamura, Institute of Plant and Microbial Biology, Academia Sinica, 128 sec.2 Academia Rd. Nankang, Taipei 11529, Taiwan TEL: +886-2-27871129 FAX: +886-2-27827954 E-mail: nakamura@gate.sinica.edu.tw

Abstract:

Phosphatidylglycerol (PG) is the exclusive phospholipid synthesized in chloroplasts and plays important roles in photosynthesis. However, phosphatidylglycerophosphate phosphatase (PGPP), which catalyzes the final step of PG biosynthesis, is a missing piece in photosynthetic eukaryotes. Here, we isolated a previously uncharacterized haloacid dehalogenase-like phosphatase, designated CrPGPP1, as a putative PGPP in *Chlamydomonas reinhardtii*. *CrPGPP1* complemented growth and lipid compositional defects in $\Delta gep4$, a yeast mutant of PGPP, which indicates that CrPGPP1 is a functional PGPP. Two aspartic acid residues, which are both essential for the yeast PGPP (Gep4p) activity, are also conserved in the putative

catalytic motif of CrPGPP1. Site-specific mutagenesis showed that the first but not the second aspartic acid residue was required for CrPGPP1 to complement the growth defect of $\Delta gep4$ mutant, which highlights the distinct molecular features of CrPGPP1. Our results suggest that CrPGPP1 is a functional PGPP in *C. reinhardtii*, for the first PGPP in photosynthetic eukaryotes.

Keywords: *Chlamydomonas reinhardtii*, chloroplast, phosphatidylglycerol, phosphatidylglycerophosphate phosphatase, photosynthesis

Abbreviations: CDP-DAG, CDP-diacylglycerol; CDS, CDP-diacylglycerol synthase; CL, cardiolipin; HAD, haloacid dehalogenase; PAH, phosphatidate phosphohydrolase; PGP, phosphatidylglycerol phosphate; PG, phosphatidylglycerol; PGPP, phosphatidylglycerophosphate phosphatase; PGPS, phosphatidylglycerophosphate synthase

1. Introduction

Phosphatidylglycerol (PG) is an anionic phospholipid class found widely in prokaryotes and eukaryotes. In higher plants, PG is the only major phospholipid class of thylakoid membranes and is a minor component of the other organelles. The biosynthetic activity of PG is found in endoplasmic reticulum (ER), mitochondria and plastids [1-3]. The PG biosynthetic pathway begins with conversion of CDP-diacylglycerol (CDP-DAG) to phosphatidylglycerol phosphate (PGP) catalyzed by PGP synthase (PGPS), which is then dephosphorylated by PGP phosphatase (PGPP) to produce PG. In mitochondria, PG is further converted to cardiolipin (CL), an exclusive phospholipid class found in the mitochondrial inner envelope.

Gene knockout study has revealed the crucial role of PG biosynthesis. Disruption of *pgsA*, encoding a PGPS in *Synechocystis* sp. PCC 6803, affects cell growth and photosynthetic activity unless PG is supplemented exogenously [4-6]. Arabidopsis possesses two PGPS, PGP1 and PGP2; knocking out *PGP1* severely affects photosynthesis but not the mitochondrial function [7-10], and double knockout of *PGP1* and *PGP2* further reduces PG levels to trace amounts, thereby affecting overall plant growth [11]. The importance of PG in mitochondria is suggested by the study of CL synthase (CLS), in that Arabidopsis CLS1 is localized at mitochondria and required for mitochondria function [12-14].

PGPP catalyzes the final reaction of PG biosynthesis (Fig. 1A), and the gene encoding a protein with PGPP activity was unknown for a long time. The first cloning of functional PGPP in a photosynthetic organism was reported in a photosynthetic prokaryote, *Anabaena* sp. PCC 7120 [15]. However, the homologs in *Synechocystis* sp.

PCC 6803 and *Arabidopsis thaliana* encode phosphatidic acid phosphatase [16], so another gene family may encode PGPP in these model organisms.

A distinct PGPP, designated Gep4p, was found recently in *Saccharomyces cerevisiae*; its defect affects mitochondrial function and CL biosynthesis [17]. Gep4p belongs to the haloacid dehalogenase (HAD)-like phosphatase superfamily. The family in *A. thaliana* features a few lipid phosphatases, such as phosphatidate phosphohydrolase (PAH1 and PAH2) and phosphoethanolamine/phosphocholine phosphatase [18,19].

Here, we used *Chlamydomonas reinhardtii* as a model organism and found that a previously uncharacterized HAD-like phosphatase gene, Cre04.g219900 (designated *CrPGPP1*), encodes a protein that shares high sequence similarity with *S. cerevisiae* Gep4p [17]. Heterologous expression of *CrPGPP1* in the $\Delta gep4$ mutant complemented the growth defect and temperature sensitivity as well as altered glycerolipid composition, which suggests that *CrPGPP1* is a functional PGPP. Moreover, the effect of site-specific mutagenesis in the conserved residues within the putative catalytic motif differed from that observed for Gep4p of *S. cerevisiae*. We suggest that *CrPGPP1* is a functional PGPP, for the first PGPP in photosynthetic eukaryotes.

2. Materials and Methods

2.1 Yeast strains and culture conditions

The strains produced in this work are described in Supplementary Table 1. Cells were grown in YPD media. Cells harboring the *URA3* marker plasmid were grown in synthetic complete medium lacking uracil (SC –Ura).

2.2 Cloning of plasmid vectors

CrPGPP1 (Cre04.g219900): To construct pCH152, a 834-bp fragment was amplified from the cDNA template of *C. reinhardtii* with the primers CH754 and CH755 and inserted into *EcoRI* and *XhoI* sites of pCH078 [20].

ScGEP4 (YHR100C): To construct pCH154, a 582-bp fragment was amplified from yeast wild type genomic DNA with the primers CH756 and CH757 and inserted into *EcoRI* and *XhoI* sites of pCH078.

CrPGPP1^{D103N}-HA: To construct pCH266, a 345-bp of 3' end fragment and a 567-bp of 5' end fragment were amplified from pCH152 using the primer sets (CH754 and CH802) and (CH801 and CH800), respectively. Next, these two fragments were mixed as a template to amplify a 861-bp full length fragment with the primers CH754 and CH800. The obtained fragment was inserted into *EcoRI* and *XhoI* sites of pCH078. The specific mutation (D103N) was confirmed by sequencing.

CrPGPP1^{D105N}-HA: To construct pCH166, a 861-bp fragment was amplified from pCH152 with the primers CH754 and CH800 and inserted into *EcoRI* and *XhoI* sites of pCH078. After the sequencing, we found that specific mutation (D105N) was created in the coding sequence of *CrPGPP1*.

CrPGPP1^{D103N, D105N}-HA: To construct pCH172, a 861-bp fragment was amplified from pCH152 with two forward primers, CH754 and CH801, and two reverse primers, CH802 and CH800. The amplified DNA fragment was inserted into *EcoRI* and *XhoI* sites of pCH078. The primer sequences and plasmids are in Supplementary Tables 2 and 3, respectively.

2.3 Lipid extraction and fatty acid analysis

Yeast cells grown to stationary phase were harvested by centrifugation (3,000 *g* for 5 min). Cell pellets were treated with pre-heated isopropanol containing 0.01% butylated hydroxytoluene at 75°C for 15 min to inactivate phospholipase activity. Total lipids were extracted as described [21]. Phospholipids were separated from total lipid extracts by 2D thin-layer chromatography (TLC) with the solvent system of chloroform/methanol/7 N ammonia; 15:10:1 (by vol) for the first dimension and chloroform/methanol/acetic acid/water; 170:20:15:3 for the second dimension [22]. Each lipid class was identified on TLC plates by primuline staining, scraped off, acyl moieties hydrolyzed and methylated to prepare fatty acid methyl esters (FAMES) with HCl-methanol solution including 1 μ M of pentadecanoic acid as an internal standard. The resulting FAMES were extracted by use of *n*-hexane and analyzed by gas chromatography (GC-2010; Shimadzu, Kyoto, Japan) with FID detector equipped with a ULBON HR-SS-10 column (Shinwa Chemical Industries, Japan). Data are mean $\pm$ SD from three biological replicates.

3. Results

3.1 Analysis of amino acid sequences of CrPGPP1

The deduced amino acid length from our cloned open reading frame of Cre04.g219900 was 269, which was significantly longer than that for Gep4p (185 amino acids). Amino acid sequence alignment showed that translated Cre04.g219900 had considerably longer extension on the N-terminal end and a short

extension on the C-terminal end (Fig. 1B). The entire sequence of Gep4p was homologous with the translated amino acid sequence of Cre04.g219900. In particular, we found an well-conserved inverted motif of typical HAD-like phosphatase [(V/T)xDxD] (Fig. 1B), which suggests that Cre04.g219900 may encode a functional phosphatase. We designated this gene as *CrPGPP1* and performed a series of functional assays to investigate its function.

3.2 Recovery of growth defect in $\Delta gep4$ mutant by transformation with *CrPGPP1*.

To investigate whether *CrPGPP1* is a functional PGPP, we performed heterologous complementation assay of the *S. cerevisiae* $\Delta gep4$ mutant. As previously reported, *GEP4* encodes a PGPP and the $\Delta gep4$ mutant shows a temperature-sensitive growth defect; the growth rate was severely affected at 30°C and cells no longer survive at 37°C [17]. We cloned the open reading frame of *CrPGPP1* into a yeast shuttle vector and transformed it into $\Delta gep4$ mutant cells. The $\Delta gep4$ mutant harboring *CrPGPP1* fully recovered cell growth at both 30°C and 37°C as did the $\Delta gep4$ mutant harboring *S. cerevisiae* *GEP4* (control cells) (Fig. 2A).

To investigate whether this functional complementation was associated with lipid composition change, we analyzed the major phospholipid composition of these strains. Compared to the wild type, $\Delta gep4$ showed increased composition of PC and PE at the expense of PS and PI (Fig. 2B). In the $\Delta gep4$ mutant harboring *CrPGPP1*, the phospholipid composition was mostly restored to that of wild type except for a slight increase in PE and decrease in PS levels. PG and CL were undetectable in both the wild type and complemented strains under our experimental conditions, which reflects that these anionic lipids are minor in abundance but crucial for growth

[23]. Taken together, these results suggest that CrPGPP1 is a functional PGPP that complements the phenotypic defect and lipid composition changes of $\Delta gep4$.

3.3 Site-specific mutagenesis of putative catalytic motif revealed a critical amino acid residue for CrPGPP1 function that is distinct from Gep4p.

To further characterize the function of CrPGPP1, we mutated the putative catalytic motif and assessed the effect of mutation in the $\Delta gep4$ mutant. CrPGPP1 and Gep4p both have an inverted HAD-like phosphatase motif, VxDxD (Fig. 1B). Previous study showed that substitution of either of the aspartic acids to asparagine abolished the complementation of the growth defect of $\Delta gep4$, which suggests that these amino acid residues are critical for the catalytic activity of Gep4p [17]. We mutated these two aspartic acids in the corresponding motif (D103N and D105N) in CrPGPP1 with a C-terminal HA tag. CrPGPP1 harboring the substituted D103N failed to complement the growth defect of $\Delta gep4$ (Fig. 3). However, the D105N substitution did not disrupt the complementation of $\Delta gep4$ by CrPGPP1, which suggests that this amino acid residue is not critical for the catalytic function in CrPGPP1. Moreover, the CrPGPP1 carrying both D103N and D105N did not complement the growth defect of $\Delta gep4$ (Fig. 3). These results suggest that only the first aspartic acid residue of CrPGPP1 is critical for the functional complementation of $\Delta gep4$ mutant. Thus, a distinct property of the phosphatase motif was revealed in CrPGPP1 as compared with yeast Gep4p.

4. Discussion

Lipid biosynthesis in eukaryotic microalgae is of increasing interest because of the high competency for carbon fixation and efficient carbon storage as neutral lipids such as triacylglycerols. Because of the high capacity of oil production and little conflict with biomass from terrestrial plants, eukaryotic algae are considered a feasible bioresource. However, genes encoding glycerolipid biosynthetic enzymes are largely uncharacterized in the model eukaryotic alga *C. reinhardtii*. Given that PG has a critical commitment to the photosynthetic function, study of the PG biosynthetic pathway in *C. reinhardtii* is highly demanded. In this study, we isolated and characterized previously unknown HAD-like phosphatase Cre04.g219900 as CrPGPP1, for the final reaction step of PG biosynthesis in *C. reinhardtii*. CrPGPP1 shares overall high amino acid sequence similarity with Gep4p, a *S. cerevisiae* ortholog of PGPP (Fig. 1B), and *CrPGPP1* complemented the growth defect and lipid composition change of the $\Delta gep4$ mutant (Fig. 2). Our results suggest that *CrPGPP1* is a functional PGPP in *C. reinhardtii*, which represents the first PGPP isolated in photosynthetic eukaryotes.

Site-specific mutagenesis analysis of the putative phosphatase motif (VxDxD) showed that the first (D103) but not the second aspartic acid (D105) was required for CrPGPP1 to complement the growth defect of $\Delta gep4$ mutant. Meanwhile, both aspartic acid residues in the motif were essential for the Gep4p function. These data highlight the distinct molecular features of CrPGPP1 from those of the yeast ortholog and suggest that the first aspartic acid of the VxDxD motif of CrPGPP may function as a catalytic center of the enzyme.

The PG biosynthetic pathway in *C. reinhardtii* is assumed to be similar to that in *Arabidopsis thaliana* (Fig. 4). First, phosphatidic acid (PA) is converted to CDP-DAG by CDP-DAG synthase (CDS). *C. reinhardtii* has two CDSs, annotated as CDS1 and CDS2 (Table 1) [24]. *A. thaliana* has two plastidic CDSs, CDS4 and CDS5, which function redundantly in chloroplasts [25] in addition to ER-localized CDS1, CDS2 and CDS3 [26]. Next, glycerol 3-phosphate is incorporated by the catalytic action of PGPS to form PGP. Here, *C. reinhardtii* has two annotated PGPS, PGP1 and PGP2 [24]. *A. thaliana* also has two PGPS, PGP1 and PGP2, with PGP1 as the major contributor to the chloroplast PG biosynthesis [7,8,11]. Finally, PGP is dephosphorylated by PGPP to produce PG, and CrPGPP1 is revealed as a functional PGPP in the current study. We found that deduced amino acid sequence of Arabidopsis At3g58830 is homologous with that of CrPGPP1, so similar sets of genes may be responsible for the biosynthesis of PG in *C. reinhardtii* and *A. thaliana*. In *C. reinhardtii*, characterization of CDS1, PGP1 and PGP2 awaits future study, which will unravel the integral PG biosynthesis in *C. reinhardtii* and provide further support for our present findings and understanding of PG biosynthesis in eukaryotic algae.

Acknowledgements

The authors thank Kazue Kanehara for critically reading the manuscript. This research was supported by CREST (to H.W) and PRESTO (to Y.N), Japan Science and Technology Agency, and the core budget of Institute of Plant and Microbial Biology, Academia Sinica (to Y.N). Y.N. is supported by EMBO Young Investigator Program.

References

- [1] J. Andrews, J.B. Mudd, Phosphatidylglycerol synthesis in pea chloroplasts: pathway and localization, *Plant Physiol* 79 (1985) 259-265.
- [2] R. Griebau, M. Frentzen, Biosynthesis of Phosphatidylglycerol in Isolated Mitochondria of Etiolated Mung Bean (*Vigna radiata* L.) Seedlings, *Plant Physiol* 105 (1994) 1269-1274.
- [3] T.S. Moore, Phosphatidylglycerol synthesis in castor bean endosperm: kinetics, requirements, and intracellular localization, *Plant Physiol* 54 (1974) 164-168.
- [4] M. Hagio, Z. Gombos, Z. Várkonyi, K. Masamoto, N. Sato, M. Tsuzuki, H. Wada, Direct evidence for requirement of phosphatidylglycerol in photosystem II of photosynthesis, *Plant Physiol* 124 (2000) 795-804.
- [5] N. Sato, M. Hagio, H. Wada, M. Tsuzuki, Requirement of phosphatidylglycerol for photosynthetic function in thylakoid membranes, *Proc Natl Acad Sci U S A* 97 (2000) 10655-10660.
- [6] Z. Gombos, Z. Várkonyi, M. Hagio, M. Iwaki, L. Kovács, K. Masamoto, S. Itoh, H. Wada, Phosphatidylglycerol requirement for the function of electron acceptor plastoquinone Q(B) in the photosystem II reaction center, *Biochemistry* 41 (2002) 3796-3802.
- [7] M. Hagio, I. Sakurai, S. Sato, T. Kato, S. Tabata, H. Wada, Phosphatidylglycerol is essential for the development of thylakoid membranes in *Arabidopsis thaliana*, *Plant Cell Physiol* 43 (2002) 1456-1464.
- [8] C. Xu, H. Härtel, H. Wada, M. Hagio, B. Yu, C. Eakin, C. Benning, The *pgp1* mutant locus of *Arabidopsis* encodes a phosphatidylglycerolphosphate synthase with impaired activity, *Plant Physiol* 129 (2002) 594-604.
- [9] E. Babiychuk, F. Müller, H. Eubel, H.P. Braun, M. Frentzen, S. Kushnir, *Arabidopsis* phosphatidylglycerolphosphate synthase 1 is essential for chloroplast differentiation, but is dispensable for mitochondrial function, *Plant J* 33 (2003) 899-909.

- [10] K. Kobayashi, S. Fujii, M. Sato, K. Toyooka, H. Wada, Specific role of phosphatidylglycerol and functional overlaps with other thylakoid lipids in Arabidopsis chloroplast biogenesis, Plant Cell Rep (2014) DOI 10.1007/s00299-014-1719-z.
- [11] R. Tanoue, M. Kobayashi, K. Katayama, N. Nagata, H. Wada, Phosphatidylglycerol biosynthesis is required for the development of embryos and normal membrane structures of chloroplasts and mitochondria in Arabidopsis, FEBS Lett 588 (2014) 1680-1685.
- [12] K. Katayama, I. Sakurai, H. Wada, Identification of an *Arabidopsis thaliana* gene for cardiolipin synthase located in mitochondria, FEBS Lett 577 (2004) 193-198.
- [13] B. Pineau, M. Bourge, J. Marion, C. Mauve, F. Gilard, L. Maneta-Peyret, P. Moreau, B. Satiat-Jeunemaître, S.C. Brown, R. De Paepe, A. Danon, The importance of cardiolipin synthase for mitochondrial ultrastructure, respiratory function, plant development, and stress responses in Arabidopsis, Plant Cell 25 (2013) 4195-4208.
- [14] R. Pan, A.D. Jones, J. Hu, Cardiolipin-mediated mitochondrial dynamics and stress response in Arabidopsis, Plant Cell 26 (2014) 391-409.
- [15] F. Wu, Z. Yang, T. Kuang, Impaired photosynthesis in phosphatidylglycerol-deficient mutant of cyanobacterium *Anabaena* sp. PCC7120 with a disrupted gene encoding a putative phosphatidylglycerophosphatase, Plant Physiol 141 (2006) 1274-1283.
- [16] Y. Nakamura, M. Tsuchiya, H. Ohta, Plastidic phosphatidic acid phosphatases identified in a distinct subfamily of lipid phosphate phosphatases with prokaryotic origin, J Biol Chem 282 (2007) 29013-29021.
- [17] C. Osman, M. Haag, F.T. Wieland, B. Brügger, T. Langer, A mitochondrial phosphatase required for cardiolipin biosynthesis: the PGP phosphatase Gep4, EMBO J 29 (2010) 1976-1987.

- [18] Y. Nakamura, R. Koizumi, G. Shui, M. Shimojima, M.R. Wenk, T. Ito, H. Ohta, Arabidopsis lipins mediate eukaryotic pathway of lipid metabolism and cope critically with phosphate starvation, *Proc Natl Acad Sci U S A* 106 (2009) 20978-20983.
- [19] A. May, M. Spinka, M. Köck, *Arabidopsis thaliana* PECP1: enzymatic characterization and structural organization of the first plant phosphoethanolamine/phosphocholine phosphatase, *Biochim Biophys Acta* 1824 (2012) 319-325.
- [20] C.H. Hung, M.Y. Ho, K. Kanehara, Y. Nakamura, Functional study of diacylglycerol acyltransferase type 2 family in *Chlamydomonas reinhardtii*, *FEBS Lett* 587 (2013) 2364-2370.
- [21] J. Folch, M. Lees, G.H. Sloane Stanley, A simple method for the isolation and purification of total lipids from animal tissues, *J Biol Chem* 226 (1957) 497-509.
- [22] Y. Nakamura, H. Arimitsu, Y. Yamaryo, K. Awai, T. Masuda, H. Shimada, K. Takamiya, H. Ohta, Digalactosyldiacylglycerol is a major glycolipid in floral organs of *Petunia hybrida*, *Lipids* 38 (2003) 1107-1112.
- [23] D.B. Ostrander, M. Zhang, E. Mileykovskaya, M. Rho, W. Dowhan, Lack of mitochondrial anionic phospholipids causes an inhibition of translation of protein components of the electron transport chain. A yeast genetic model system for the study of anionic phospholipid function in mitochondria, *J Biol Chem* 276 (2001) 25262-25272.
- [24] W.R. Riekhof, B.B. Sears, C. Benning, Annotation of genes involved in glycerolipid biosynthesis in *Chlamydomonas reinhardtii*: discovery of the betaine lipid synthase BTA1_{Cr}, *Eukaryot Cell* 4 (2005) 242-252.
- [25] A. Haselier, H. Akbari, A. Weth, W. Baumgartner, M. Frentzen, Two closely related genes of Arabidopsis encode plastidial cytidinediphosphate diacylglycerol synthases essential for photoautotrophic growth, *Plant Physiol* 153 (2010) 1372-1384.

[26] Y. Zhou, H. Peisker, A. Weth, W. Baumgartner, P. Dörmann, M. Frentzen, Extraplasmidial cytidinediphosphate diacylglycerol synthase activity is required for vegetative development in *Arabidopsis thaliana*, Plant J 75 (2013) 867-879.

Figure legends:

Fig. 1. Identification of PGPP in *Chlamydomonas reinhardtii*. (A) Chemical structure of phosphatidylglycerol phosphate and the dephosphorylation reaction catalyzed by PGPP. (B) Amino acid sequence alignment of *C. reinhardtii* PGPP1 (CrPGPP1) with *Saccharomyces cerevisiae* Gep4p. Domains highlighted in bold indicate that no counterpart is found in Gep4p. A putative inverted phosphatase motif is underlined and amino acid residues mutated in this study are marked with asterisks.

Fig. 2. Heterologous complementation of temperature-sensitive growth defect and lipid compositional change in the *Saccharomyces cerevisiae* $\Delta gep4$ mutant with CrPGPP1. (A) Culture of the wild-type, $\Delta gep4$ mutant and $\Delta gep4$ mutant harboring *S. cerevisiae* GEP4 or CrPGPP1 involved serial 10-fold dilution from left to right starting at OD₆₀₀ 0.1; 5 μ L each was spotted onto YPD media and incubated for 2 days at 30°C or 37°C. Image is representative of 3 replicates. (B) Phospholipid content of wild-type, $\Delta gep4$ mutant and $\Delta gep4$ mutant harboring CrPGPP1. Total lipids were extracted from cells grown to stationary phase, separated by 2D TLC and content of phospholipids was quantified by gas chromatography. Data are mean $\pm$ SD from 3 biological replicates. Asterisks show significance (*, $p < 0.05$; **, $p < 0.01$) by Student's *t*-test.

Fig. 3. Heterologous complementation of temperature-sensitive growth defect in the *Saccharomyces cerevisiae* $\Delta gep4$ mutant with HA-tagged CrPGPP1 harboring amino acid

substitution. Culture of the wild-type, $\Delta gep4$ mutant and $\Delta gep4$ mutant harboring C-terminally HA-tagged *CrPGPP1* with amino acid substitution (D103N, D105N or D105N with D103N) involved serial 10-fold dilution from left to right starting at OD₆₀₀ 0.1; 5 μ L each was spotted onto YPD media and incubated for 2 days at 30°C or 37°C. Image is representative of 3 replicates.

Fig. 4. Proposed PG biosynthetic pathway and genes encoding enzymes for each reaction step in *Chlamydomonas reinhardtii* and *Arabidopsis thaliana*. See Table 1 for the details of these genes. CDP-DAG, CDP-diacylglycerol; CDS, CDP-diacylglycerol synthase; PA, phosphatidic acid; PG, phosphatidylglycerol; PGP, phosphatidylglycerol phosphate; PGPP, phosphatidylglycerophosphate phosphatase.

Table 1. List of genes involved in PG biosynthesis in *Chlamydomonas reinhardtii* and *Arabidopsis thaliana*.

Table 1 List of genes involved in PG biosynthesis in *Chlamydomonas reinhardtii* and *Arabidopsis thaliana*.

Species	Gene name	Gene ID	Molecular mass (kDa)	References
<i>C. reinhardtii</i>	<i>CrCDS1</i>	Cre03.g186200	51.2	-
	<i>CrCDS2</i>	Cre12.g489050	39.3	-
	<i>CrPGP1</i>	Cre03.g162600	32.1	-
	<i>CrPGP2</i>	Cre02.g095106	28.7	-
	<i>CrPGPP1</i>	Cre04.g219900	28.6	This work
<i>A. thaliana</i>	<i>AtCDS1</i>	At1g62430	48.7	[25,26]
	<i>AtCDS2</i>	At4g22340	48.2	[25,26]
	<i>AtCDS3</i>	At4g26770	54.9	[25,26]
	<i>AtCDS4</i>	At2g45150	46.8	[25]
	<i>AtCDS5</i>	At3g60620	43.3	[25]
	<i>AtPGP1</i>	At2g39290	32.2	[7, 8]
	<i>AtPGP2</i>	At3g55030	25.2	[11]
	<i>AtPGPP1</i>	At3g58830	38.5	-

B

Cre04.g219900 Gep4p	1	MRSVPGPSPPCTRSLAHS CRAAARGPCGSARPRARSVSARAHSSSEASDMARVQQNFNSAG
	1	-----MNISGTLNTLR-----
Cre04.g219900 Gep4p	61	VGLFFSLFGGNQSLALPHLAAPDIRHVDWRALKAAGFKGLVFDKDNLTSLPFALEVERL
	12	-----LLYNPSLCKPSLVVPTFN--DLPIPIHDSIKAVVLDKDNCTAFPHDDKIWDY
Cre04.g219900 Gep4p	121	QPALAGCLEAFGGRAVLYSNSAGLQQYDPEGKEAAALEAALGIPVLRHADKKPGGGCABL
	63	LQHWETLRSKYSNKALLIVSNTAGSNSDKDYSQAKLLEDKGTIPVLRHSTKKPGCHNEIL
Cre04.g219900 Gep4p	181	EAHFG---CPAPQLIMVGDRYLTDIAFGNRHGMLTVHVQPLTTSGEFFGVVMARRIEEF
	123	DYFYRNKTITNPKEVAVVGDRLFTDILMANLMGSYGWVIRDGVK-----VSANPLSKF
Cre04.g219900 Gep4p	237	WVARWTSFGVHPPAHS LAPHDTLAAYVKDQPIA
	176	EKKLYNFLGF-----

A

B

Hung et al, 2015 Fig.3

<i>Chlamydomonas reinhardtii</i>	CDS1 CDS2	PGP1 PGP2	PGPP1
<i>Arabidopsis thaliana</i>	CDS1 CDS2 CDS3 CDS4 CDS5	PGP1 PGP2	PGPP1