



# Functional Specificity of Cardiolipin Synthase Revealed by the Identification of a Cardiolipin Synthase CrCLS1 in *Chlamydomonas reinhardtii*

Chun-Hsien Hung<sup>1</sup>, Koichi Kobayashi<sup>2</sup>, Hajime Wada<sup>2,3</sup> and Yuki Nakamura<sup>1,4\*</sup>

<sup>1</sup> Institute of Plant and Microbial Biology, Academia Sinica, Taipei, Taiwan, <sup>2</sup> Department of Life Sciences, Graduate School of Arts and Sciences, The University of Tokyo, Tokyo, Japan, <sup>3</sup> Japan Science and Technology Agency, CREST, Saitama, Japan, <sup>4</sup> Japan Science and Technology Agency, PRESTO, Saitama, Japan

## OPEN ACCESS

### Edited by:

Weiwen Zhang,  
Tianjin University, China

### Reviewed by:

Alberto A. Iglesias,  
Instituto de Agrobiotecnología del  
Litoral – Consejo Nacional  
de Investigaciones Científicas y  
Tecnológicas, Argentina  
Takashi Osanai,  
Meiji University, Japan  
Song Xue,  
Marine Bioengineering Group  
Department of Biotechnology Dalian  
Institute of Chemical Physics Chinese  
Academy of Sciences, China

### \*Correspondence:

Yuki Nakamura  
nakamura@gate.sinica.edu.tw

### Specialty section:

This article was submitted to  
Microbiotechnology, Ecotoxicology  
and Bioremediation,  
a section of the journal  
Frontiers in Microbiology

**Received:** 15 October 2015

**Accepted:** 21 December 2015

**Published:** 12 January 2016

### Citation:

Hung C-H, Kobayashi K, Wada H  
and Nakamura Y (2016) Functional  
Specificity of Cardiolipin Synthase  
Revealed by the Identification of a  
Cardiolipin Synthase CrCLS1  
in *Chlamydomonas reinhardtii*.  
Front. Microbiol. 6:1542.  
doi: 10.3389/fmicb.2015.01542

Phosphatidylglycerol (PG) and cardiolipin (CL) are two essential classes of phospholipid in plants and algae. Phosphatidylglycerophosphate synthase (PGPS) and cardiolipin synthase (CLS) involved in the biosynthesis of PG and CL belong to CDP-alcohol phosphotransferase and share overall amino acid sequence homology. However, it remains elusive whether PGPS and CLS are functionally distinct *in vivo*. Here, we report identification of a gene encoding CLS in *Chlamydomonas reinhardtii*, CrCLS1, and its functional compatibility. Whereas CrCLS1 did not complement the growth phenotype of a PGPS mutant of *Synechocystis* sp. PCC 6803, it rescued the temperature-sensitive growth phenotype, growth profile with different carbon sources, phospholipid composition and enzyme activity of  $\Delta crd1$ , a CLS mutant of *Saccharomyces cerevisiae*. These results suggest that CrCLS1 encodes a functional CLS of *C. reinhardtii* as the first identified algal CLS, whose enzyme function is distinct from that of PGPSs from *C. reinhardtii*. Comparison of CDP-alcohol phosphotransferase motif between PGPS and CLS among different species revealed a possible additional motif that might define the substrate specificity of these closely related enzymes.

**Keywords:** CDP-alcohol phosphotransferase, cardiolipin, cardiolipin synthase, phosphatidylglycerol, PGPS, *Chlamydomonas reinhardtii*, *Synechocystis* sp. PCC 6803, *Saccharomyces cerevisiae*

## INTRODUCTION

Functional specificity of an enzyme is crucial in keeping metabolic reactions in order. This largely relies on the substrate specificity defined by the catalytic motif. Thus, enzymes are often categorized into groups according to the existence of common catalytic motif(s). In phospholipid metabolism, a number of important reaction steps are catalyzed by CDP-alcohol phosphotransferases (Li-Beisson et al., 2013, 2015). These include CLS, PGPS, phosphatidylinositol (PI) synthase, phosphatidylserine (PS) synthase, phosphatidylcholine (PC)

**Abbreviations:** CDP, cytidine 5'-diphosphate; CL, cardiolipin; CLS, cardiolipin synthase; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PGP, phosphatidylglycerol phosphate; PGPP, phosphatidylglycerophosphate phosphatase; PGPS, phosphatidylglycerophosphate synthase; PI, phosphatidylinositol; PS, phosphatidylserine.

synthase, and aminoalcohol phosphotransferase for the biosynthesis of CL, PG, PI, PS, PC, and PE, respectively. Because these are the major phospholipid classes found in diverse organisms from bacteria to mammals and seed plants, it can be stated that CDP-alcohol phosphotransferases are crucial in the entire phospholipid metabolism.

In plants and algae, PG is an indispensable phospholipid class in photosynthetic function (Hagio et al., 2000, 2002; Sato et al., 2000; Babiychuk et al., 2003; Yu and Benning, 2003). Moreover, CL, which is an anionic phospholipid class widely distributed in different kingdom and found exclusively at the inner membrane of mitochondria (Lewis and McElhaney, 2009), has an essential role in mitochondrial function and thus plant growth (Katayama et al., 2004; Pineau et al., 2013). The biosynthesis of these lipid classes begins with the conversion of phosphatidic acid (PA) into CDP-diacylglycerol (CDP-DAG) by CDP-DAG synthase (CDS; Sato et al., 2000; Haselier et al., 2010; Zhou et al., 2013). Next, PGPS converts CDP-DAG to phosphatidylglycerol phosphate (PGP), which is dephosphorylated by PGP phosphatase (PGPP) to produce PG (Muller and Frentzen, 2001; Hagio et al., 2000, 2002; Wu et al., 2006; Osman et al., 2010; Hung et al., 2015b). Furthermore, PG is converted to CL by CLS in mitochondria (Kadenbach et al., 1982; Jiang et al., 1997, 1999). Initially, 3 PGPSs were proposed in *Arabidopsis thaliana* (PGP1, PGP2, and PGP3) based on the amino acid sequence similarity (Xu et al., 2002). However, the third isoform (PGP3) was later shown not to be a functional PGPS but instead functions as CLS (Katayama et al., 2004). Subsequent gene knockout studies defined distinct *in vivo* function of CLS associated with mitochondrial function (Pineau et al., 2013). Thus, PGPS and CLS are functionally independent, although they are homologous and belong to the same CDP-alcohol phosphotransferase family in *A. thaliana*. Recently, we identified and characterized genes for PGPS of *Chlamydomonas reinhardtii* (Hung et al., 2015a). We demonstrated two functional PGPS isoforms; however, genome-wide search identified an additional PGPS homolog, which is more homologous with CLS. This reminded us of the case in *A. thaliana* described above. Because reciprocal genetic complementation was not performed yet in *A. thaliana* or any other model organisms, it remains elusive whether PGPS and CLS are functionally distinct *in vivo*.

In this report, we identified the additional homolog of PGPS in *C. reinhardtii*, designated *CrCLS1* (*Cre13.g604700*), and performed reciprocal functional complementation assay using *pgsA*, a PGPS mutant of *Synechocystis* sp. PCC 6803 and  $\Delta$ *crd1*, a CLS mutant of *Saccharomyces cerevisiae*. The result of functional complementation in these mutants, along with phenotype observation, lipid analysis and enzyme activity assay, demonstrated that *CrCLS1* encodes a functional CLS but not PGPS. We compared sequence similarity in detail between PGPS and CLS and noted some difference adjacent to the defined CDP-OH-P motif. Our results suggest non-overlapping function of PGPS and CLS, through the identification and characterization of CLS in *C. reinhardtii* as the first report of CLS in algae.

## MATERIALS AND METHODS

### Strains

The strains produced in this work are listed in Supplementary Table S1.

### Protein Sequence Analysis

The multiple alignment of protein sequences was performed by use of CLUSTALW<sup>1</sup>. The mitochondrial targeting sequence was predicted by use of the subcellular localization program MitoProtII<sup>2</sup> (Claros and Vincens, 1996).

### Cloning of Plasmid Vectors

*CrCLS1* (*Cre13.g604700*): To construct pCH069, a 1,060-bp fragment was amplified from the cDNA template of *C. reinhardtii* strain CC-503 (cw92 mt+) with the primers CH227 and CH228, and cloned into pENTR/D-TOPO. Then, to construct pCH178, the open reading frame (ORF) of *CrCLS1* was amplified from pCH069 with the primers CH831 and CH832, and inserted into *Xba*I and *Eco*RI sites of pCH078 (Hung et al., 2013). To construct pCH158, the ORF of *CrCLS1* was amplified from pCH069 with the primers CH776 and CH777 and inserted into *Nde*I and *Hpa*I sites of pTCP2031V (Satoh et al., 2001). The primers and plasmids used in this study are described in Supplementary Tables S2 and 3, respectively.

### Complementation Assay of the *Synechocystis* sp. PCC 6803 *pgsA* Mutant by *CrCLS1*

Complementation assay of the *Synechocystis* sp. PCC 6803 *pgsA* mutant by *CrCLS1* (pCH158) was performed as described previously (Hung et al., 2015a).

### Complementation Assay of the *S. cerevisiae* $\Delta$ *crd1* Mutant by *CrCLS1*

Complementation assay of the *S. cerevisiae*  $\Delta$ *crd1* mutant by *CrCLS1* (pCH178) was performed as described previously (Hung et al., 2015a).

### Lipid Extraction and Analysis

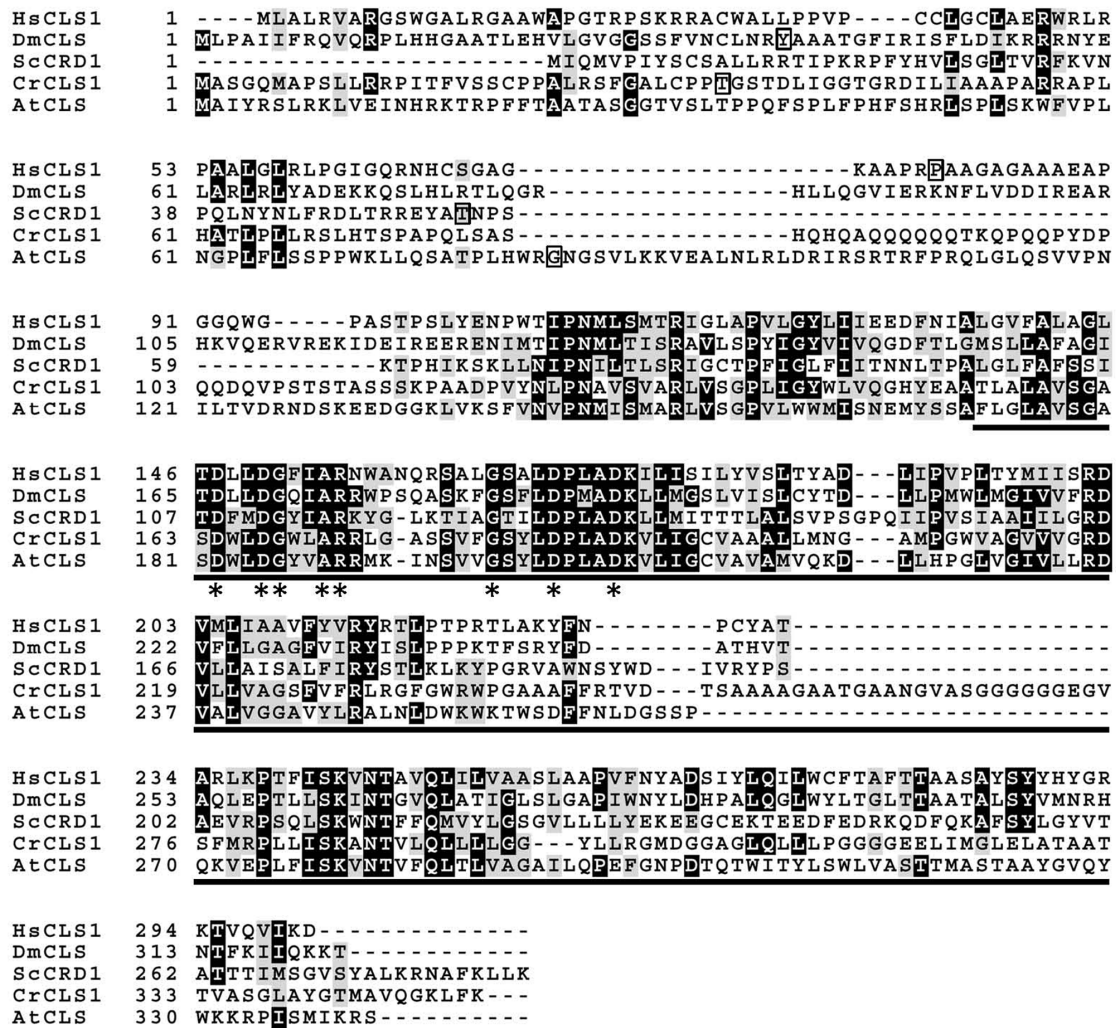
Lipid extraction and analysis were performed as previously described (Hung et al., 2013) except that 2D thin-layer chromatography (TLC) was used to separate phospholipid classes with the solvent system of chloroform/methanol/7 N ammonia 120:80:8 (by vol) for the first dimension and chloroform/methanol/acetic acid/water 170:20:15:3 (by vol) for the second dimension (Nakamura et al., 2003).

### Radiolabeling Assay of CLS Activity

Logarithmically growing cells were resuspended in 5 ml SC-Ura medium at the cell density (OD<sub>600</sub> of 5) with 30  $\mu$ Ci KH<sub>2</sub><sup>32</sup>PO<sub>4</sub> (PerkinElmer). After shaking incubation for 8 h at room temperature, lipids were extracted from cells by the method

<sup>1</sup><http://www.genome.jp/tools/clustalw/>

<sup>2</sup><http://ihg.gsf.de/ihg/mitoprot.html>



**FIGURE 1 |** Multiple amino acid sequence alignment of *Chlamydomonas reinhardtii* CLS1 (CrCLS1) with other known CLSs, *Saccharomyces cerevisiae* CRD1 (ScCRD1), *Homo sapiens* CLS1 (HsCLS1), *Arabidopsis thaliana* CLS (AtCLS), and *Drosophila melanogaster* CLS (DmCLS). The region conserved among proteins containing a CDP-OH-P motif (PF01066.9) is underlined. Asterisks indicate the amino acid residues conserved in all sequences of proteins with the CDP-OH-P motif. Square frames indicate the terminal amino acid residues of the predicted cleavage site of putative N-terminal mitochondrial targeting sequence.

of Bligh and Dyer (1959). Lipids spotted on a TLC plate (Silica gel 60G, Merck) were developed with chloroform/methanol/acetic acid (65:25:8, v/v/v) (Haselier et al., 2010) along with PG and CL (Sigma–Aldrich) as standards and radioactive spots were visualized by Imaging Plate (Fuji Film) and BAS-2500 (GE Healthcare). Unlabeled PG and CL were stained with 0.01% primuline in 80% (v/v) acetone and detected under UV light.

### RNA Extraction and cDNA Synthesis

RNA extraction and cDNA synthesis were performed as previously described (Hung et al., 2013).

### Quantitative RT-PCR

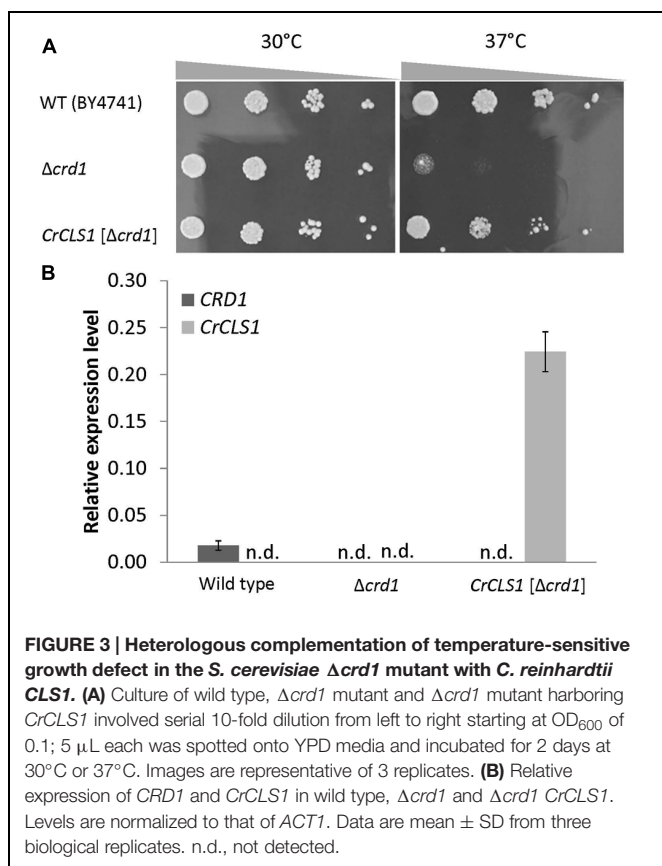
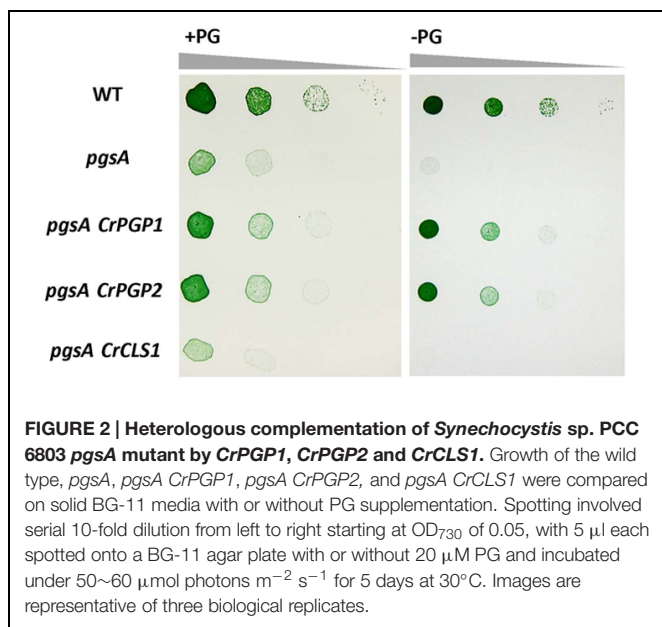
Quantitative RT-PCR analysis involved the ABI 7500 Real Time PCR System (Applied Biosystems) with the specific

oligonucleotide primer sets, CH955 and CH956, CH957 and CH958, and CH531 and CH532, for *CrCLS1*, *CRD1*, and *ACT1*, respectively. Gene expression was normalized to that of *ACT1*. Data were averaged by three technical replicates in the same run and three biological replicates in separate runs. The primer sequences are described in Supplementary Table S2.

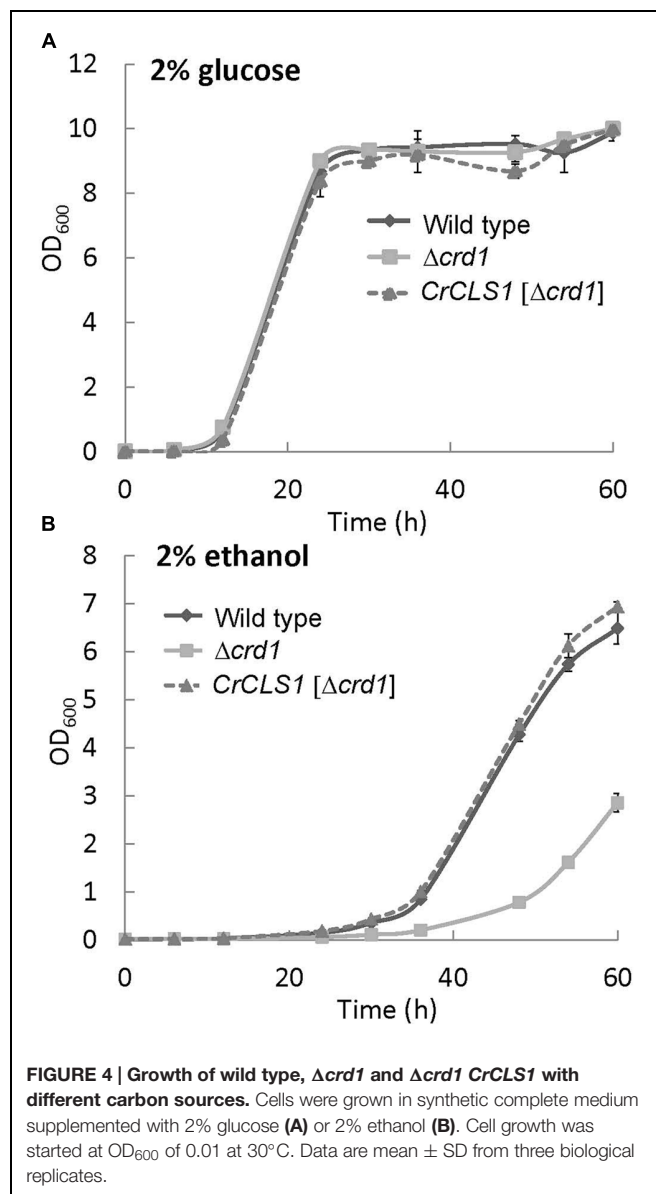
## RESULTS

### Sequence Analysis of CrCLS1

To compare the amino acid sequence similarity of the putative CrCLS1 with other known CLSs in different organisms, the deduced amino acid sequence of CrCLS1 was compared with those of *S. cerevisiae* CRD1, *Homo sapiens* CLS1,



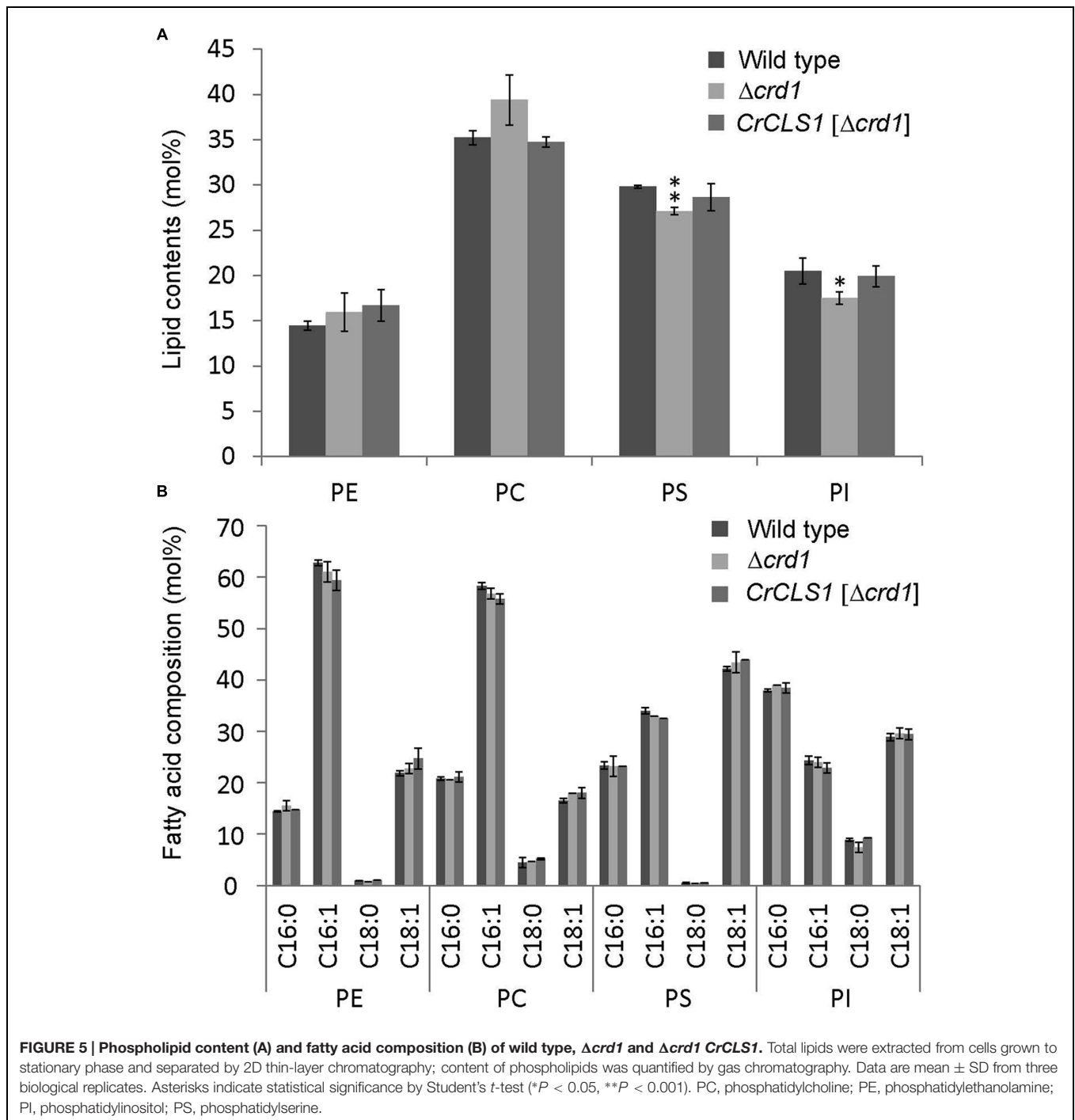
*A. thaliana* CLS, and *Drosophila melanogaster* CLS, which are functionally characterized CLS (Figure 1) (Tuller et al., 1998; Katayama et al., 2004; Chen et al., 2006; Acehan et al., 2011). In Figure 1, the region containing the CDP-OH-P motif D(X)<sub>2</sub>DG(X)<sub>2</sub>AR(X)<sub>8-9</sub>G(X)<sub>3</sub>D(X)<sub>3</sub>D is underlined and



asterisks indicate the conserved eight amino acid residues. All eight amino acids were conserved in *CrCLS1*, which suggests that *CrCLS1* encodes a functional CLS. In addition, *CrCLS1* contained a putative N-terminal mitochondrial targeting sequence predicted by the subcellular localization program MitoProtII, suggesting a possible localization of *CrCLS1* in mitochondria, where CL is exclusively localized.

### Complementation of *pgsA* by *CrCLS1*

To examine whether *CrCLS1* functions as PGPS, we transformed *CrCLS1* into the *pgsA* mutant of *Synechocystis* sp. PCC 6803, which abolishes PGPS activity and thus requires exogenous supplementation of PG for growth (Hagio et al., 2000). As shown in Figure 2, whereas the *CrPGP1* and *CrPGP2* functionally complemented the lethal phenotype of the *pgsA* mutant as reported previously (Hung et al., 2015a), *CrCLS1* failed to

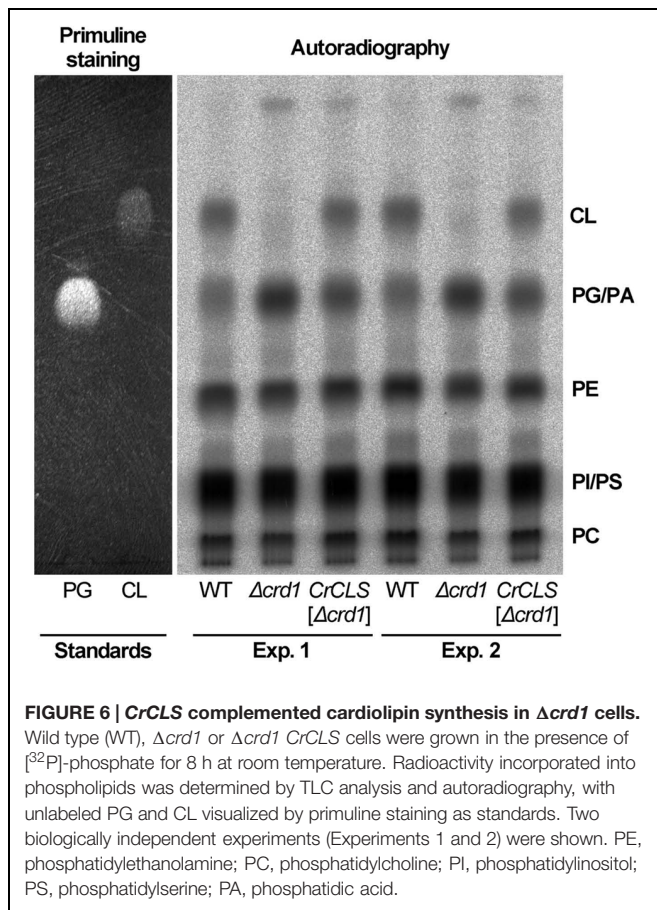


complement the growth phenotype, showing the rescued growth only in the presence of PG. Therefore, *CrCLS1* does not function as a PGPS *in vivo* in *Synechocystis* sp. PCC 6803.

### Recovery of Growth Defect in the $\Delta crd1$ Mutant Complemented by *CrCLS1*

To investigate whether *CrCLS1* encodes a functional CLS, we performed a heterologous complementation assay with the

*S. cerevisiae*  $\Delta crd1$  mutant, because *Synechocystis* sp. PCC 6803 does not contain CL and no other CLS mutant is known in algae. As previously reported, *Crd1p* has CLS activity and  $\Delta crd1$  mutant cells show a temperature-sensitive growth defect, severe at 37°C but not at 30°C (Jiang et al., 1999). The temperature-sensitive phenotype of  $\Delta crd1$  mutant cells was rescued by heterologous complementation of *HsCLS1* (Houtkooper et al., 2006), so we used this approach to investigate the function of *CrCLS1*. We cloned the ORF of *CrCLS1* into a yeast shuttle



vector and transformed it into  $\Delta crd1$  mutant cells. The  $\Delta crd1$  mutant harboring *CrCLS1* fully recovered cell growth at 37°C, whereas the  $\Delta crd1$  mutant alone showed a growth defect at this temperature (Figure 3A). Therefore, *CrCLS1* complemented the

temperature-sensitive phenotype of  $\Delta crd1$ , which suggests that *CrCLS1* encodes a functional CLS of *C. reinhardtii*.

### Expression of *CrCLS1*

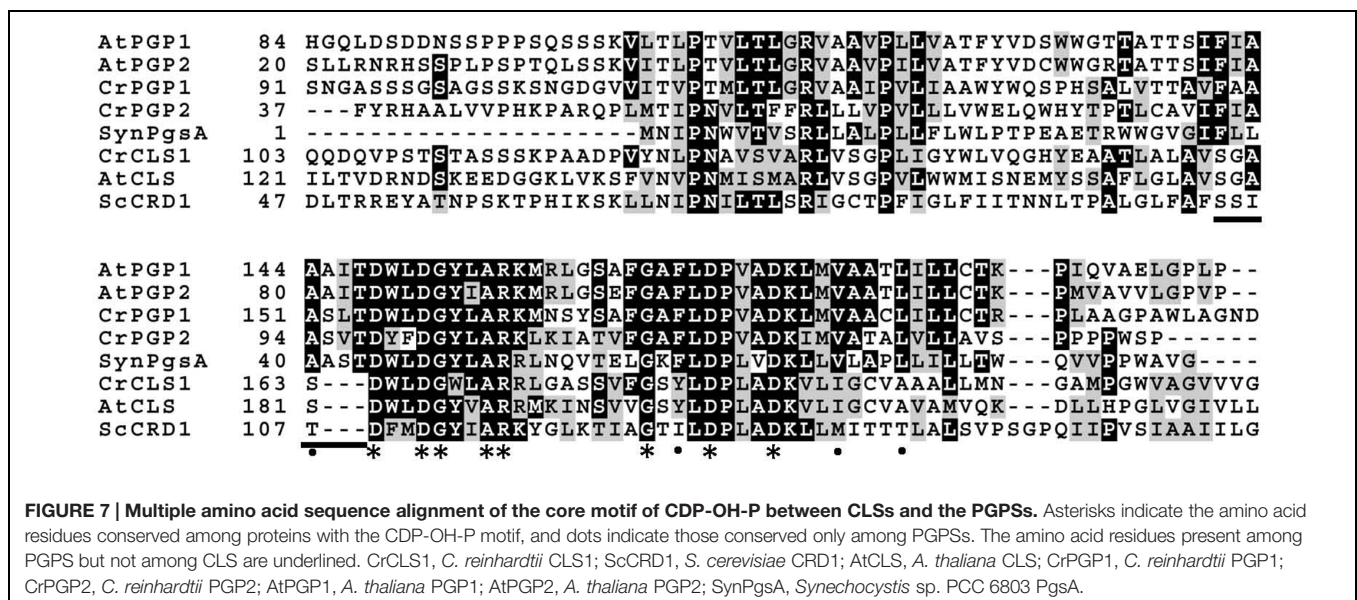
To investigate whether *CrCLS1* is appropriately expressed in the  $\Delta crd1$  mutant, we analyzed the gene expression of *CrCLS1* in the  $\Delta crd1$  mutant harboring *CrCLS1*. The relative gene expression of *CrCLS1* was 12.6-fold higher in the  $\Delta crd1$  mutant harboring *CrCLS1* than *CRD1* in the wild type (Figure 3B). Thus, *CrCLS1* is sufficiently expressed in  $\Delta crd1$  mutant cells, which supports the functional complementation shown in Figure 3A.

### Effect of Different Carbon Sources on the Growth of $\Delta crd1$ Mutant Complemented by *CrCLS1*

A previous study showed that the growth of the  $\Delta crd1$  mutant under aerobic conditions was affected with ethanol used as the sole carbon source (Tuller et al., 1998). To investigate whether the  $\Delta crd1$  mutant harboring *CrCLS1* rescued the growth defect under this condition, cells were grown in synthetic complete medium supplemented with 2% glucose or 2% ethanol as the sole carbon source. The growth rates of both the  $\Delta crd1$  mutant and  $\Delta crd1$  harboring *CrCLS1* were indistinguishable from that of wild type in 2% glucose medium (Figure 4A). However, with 2% ethanol medium, the  $\Delta crd1$  mutant harboring *CrCLS1* fully restored the growth phenotype to that of the wild type, whereas the  $\Delta crd1$  mutant showed growth retardation, as reported (Tuller et al., 1998) (Figure 4B). Therefore, *CrCLS1* could complement the growth defect of the  $\Delta crd1$  mutant with ethanol supplementation as the carbon source.

### Lipid Contents of the $\Delta crd1$ Mutant Complemented by *CrCLS1*

The phospholipid profiles of the  $\Delta crd1$  mutant were previously analyzed by radiolabeling (Tuller et al., 1998)



or mass spectrometry (Zhang et al., 2003). However, whether the  $\Delta crd1$  mutant alters the composition of major membrane phospholipid classes remained unclear. To investigate whether the complementation of the growth defect observed in **Figures 3** and **4** is associated with lipid compositional change, we analyzed the major phospholipid composition of these strains. The  $\Delta crd1$  mutant showed an increase in PC content and decrease in PS and PI contents as compared with the wild type (**Figure 5A**). In the  $\Delta crd1$  mutant harboring *CrCLS1*, phospholipid composition was restored to a level similar to that of the wild type. The fatty acid composition of PE, PC, and PI was similar among the three strains (**Figure 5B**). Thus, *CrCLS1* encodes a functional CLS that complements lipid compositional changes in the  $\Delta crd1$  mutant.

### Enzyme Activity of *CrCLS1* Expressed in the $\Delta crd1$

To investigate whether *CrCLS1* encodes a functional CLS to restore the CL synthesis defect in the  $\Delta crd1$ , we performed radiolabeling assay to analyze CLS activity. As shown in **Figure 6**, the  $\Delta crd1$  mutant harboring *CrCLS1* recovered radiolabeled spot that co-migrates with the commercial standard of CL, which is present in wild type but absent in the  $\Delta crd1$  mutant, demonstrating that the activity of CLS was recovered in the mutant harboring *CrCLS1*. Thus, *CrCLS1* encodes a functional CLS that complements CL synthesis defect of  $\Delta crd1$  mutant.

## DISCUSSION

Present study reported identification of a *CLS* gene in *C. reinhardtii*, *CrCLS1*, and examined its *in vivo* function by heterologous complementation of *pgsA*, a PGPS mutant of *Synechocystis* sp. PCC 6803, and  $\Delta crd1$ , a CLS mutant of *S. cerevisiae*. Whereas *CrCLS1* did not complement the growth phenotype of *pgsA*, it rescued the temperature-sensitive growth phenotype, growth profile with different carbon sources, phospholipid composition and enzyme activity of  $\Delta crd1$  of *S. cerevisiae*. These results suggest that *CrCLS1* is a functional gene for CLS of *C. reinhardtii* as the first identified algal CLS, which is functionally incompatible with PGPS despite their sequence homology.

Physiological roles of *CrCLS1* in *C. reinhardtii* are not reported yet; however, several transcriptomic studies have shown gene expression profiles in response to environmental stresses. For example, expression of *CrCLS1* is down-regulated in response to the deprivation of iron (Urzica et al., 2013) and nitrogen (Goodenough et al., 2014). Conversely, an upregulation is seen by copper deficiency (Castruita et al., 2011) and singlet oxygen stress (Wakao et al., 2014). These data suggest possible roles of *CrCLS1* in adaptation to circumvent environmental stresses.

Given that both PGPS and CLS belong to the CDP-alcohol phosphotransferase family and the relevant CDP-OH-P motifs are closely related (Katayama et al., 2004), what defines substrate specificity of these enzymes?

Recently, structural basis for catalysis in a CDP-alcohol phosphotransferase was revealed by crystallographic analysis (Sciara et al., 2014). According to this structure, conserved amino acid residues in the CDP-OH-P motif are associated with CDP-DAG. Since CDP-DAG is the common substrate between CLS and PGPS, this study suggests that an additional motif recognizes the other substrate (PG for CLS; glycerol 3-phosphate for PGPS). We aligned the amino acid sequences of core CDP-OH-P motif among three CLSs (*C. reinhardtii* cardiopin synthase 1, *CrCLS1*; *S. cerevisiae* CRD1, *ScCRD1*; *A. thaliana* CLS, *AtCLS*) and five PGPSs (*C. reinhardtii* PGP1, *CrPGP1*; *C. reinhardtii* PGP2, *CrPGP2*; *A. thaliana* PGP1, *AtPGP1*; *A. thaliana* PGP2, *AtPGP2*; *Synechocystis* sp. PCC 6803 *PgsA*, *SynPgsA*) (**Figure 7**). While the eight amino acid residues of the core CDP-OH-P motif  $D(X)_2DG(X)_2AR(X)_{8-9}G(X)_3D(X)_3D$  indicated by asterisks in **Figure 7** were conserved between the PGPS and CLS, we noted that seven amino acids (FxxAxxT) immediately before the core CDP-OH-P motif were highly conserved among PGPSs but not CLSs (underlined in **Figure 7**). In addition, we found additional four amino acid residues that were conserved among PGPS but not in CLS (indicated by dots in **Figure 7**). It is possible that these additional residues may define the substrate specificity between PGPS and CLS. Detailed structural analysis as well as enzymatic characterization of these residues are anticipated to experimentally validate this proposal.

## CONCLUSION

We suggest functional specificity of CLS by the identification and characterization of a CLS, *CrCLS1*, in *C. reinhardtii*.

## AUTHOR CONTRIBUTIONS

KK, HW, and YN conceived research. KK and C-HH performed experiments and analyzed data. All authors wrote and commented on the manuscript and approved the contents.

## ACKNOWLEDGMENTS

We thank Masahiro Ikeuchi (the University of Tokyo, Tokyo, Japan) for the supply of pTCP2031V. This research was supported by CREST (to HW) and PRESTO (to YN), Japan Science and Technology Agency, and the core budget of Institute of Plant and Microbial Biology, Academia Sinica (to YN).

## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <http://journal.frontiersin.org/article/10.3389/fmicb.2015.01542>

## REFERENCES

- Acehan, D., Malhotra, A., Xu, Y., Ren, M., Stokes, D. L., and Schlame, M. (2011). Cardiolipin affects the supramolecular organization of ATP synthase in mitochondria. *Biophys. J.* 100, 2184–2192. doi: 10.1016/j.bpj.2011.03.031
- Babiychuk, E., Muller, F., Eubel, H., Braun, H. P., Frentzen, M., and Kushnir, S. (2003). *Arabidopsis* phosphatidylglycerophosphate synthase 1 is essential for chloroplast differentiation, but is dispensable for mitochondrial function. *Plant J.* 33, 899–909. doi: 10.1046/j.1365-3113X.2003.01680.x
- Bligh, E. G., and Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* 37, 911–917. doi: 10.1139/c59-099
- Castruita, M., Casero, D., Karpowicz, S. J., Kropat, J., Vieler, A., Hsieh, S. I., et al. (2011). Systems biology approach in *Chlamydomonas* reveals connections between copper nutrition and multiple metabolic steps. *Plant Cell* 23, 1273–1292. doi: 10.1105/tpc.111.084400
- Chen, D., Zhang, X. Y., and Shi, Y. (2006). Identification and functional characterization of hCLS1, a human cardiolipin synthase localized in mitochondria. *Biochem. J.* 398, 169–176. doi: 10.1042/BJ20060303
- Claros, M. G., and Vincens, P. (1996). Computational method to predict mitochondrially imported proteins and their targeting sequences. *Eur. J. Biochem.* 241, 779–786. doi: 10.1111/j.1432-1033.1996.00779.x
- Goodenough, U., Blaby, I., Casero, D., Gallaher, S. D., Goodson, C., Johnson, S., et al. (2014). The path to triacylglyceride obesity in the sta6 strain of *Chlamydomonas reinhardtii*. *Eukaryot. Cell* 13, 591–613. doi: 10.1128/EC.00013-14
- Hagio, M., Gombos, Z., Varkonyi, Z., Masamoto, K., Sato, N., Tsuzuki, M., et al. (2000). Direct evidence for requirement of phosphatidylglycerol in photosystem II of photosynthesis. *Plant Physiol.* 124, 795–804. doi: 10.1104/pp.124.2.795
- Hagio, M., Sakurai, I., Sato, S., Kato, T., Tabata, S., and Wada, H. (2002). Phosphatidylglycerol is essential for the development of thylakoid membranes in *Arabidopsis thaliana*. *Plant Cell Physiol.* 43, 1456–1464. doi: 10.1093/pcp/pcf185
- Haselier, A., Akbari, H., Weth, A., Baumgartner, W., and Frentzen, M. (2010). Two closely related genes of *Arabidopsis* encode plastidial cytidinediphosphate diacylglycerol synthases essential for photoautotrophic growth. *Plant Physiol.* 153, 1372–1384. doi: 10.1104/pp.110.156422
- Houtkooper, R. H., Akbari, H., van Lenthe, H., Kulik, W., Wanders, R. J., Frentzen, M., et al. (2006). Identification and characterization of human cardiolipin synthase. *FEBS Lett.* 580, 3059–3064. doi: 10.1016/j.febslet.2006.04.054
- Hung, C. H., Endo, K., Kobayashi, K., Nakamura, Y., and Wada, H. (2015a). Characterization of *Chlamydomonas reinhardtii* phosphatidylglycerophosphate synthase in *Synechocystis* sp. PCC 6803. *Front. Microbiol.* 6:842. doi: 10.3389/fmicb.2015.00842
- Hung, C. H., Kobayashi, K., Wada, H., and Nakamura, Y. (2015b). Isolation and characterization of a phosphatidylglycerophosphate phosphatase1, PGPP1, in *Chlamydomonas reinhardtii*. *Plant Physiol. Biochem.* 92, 56–61. doi: 10.1016/j.plaphy.2015.04.002
- Hung, C. H., Ho, M. Y., Kanehara, K., and Nakamura, Y. (2013). Functional study of diacylglycerol acyltransferase type 2 family in *Chlamydomonas reinhardtii*. *FEBS Lett.* 587, 2364–2370. doi: 10.1016/j.febslet.2013.06.002
- Jiang, F., Gu, Z., Granger, J. M., and Greenberg, M. L. (1999). Cardiolipin synthase expression is essential for growth at elevated temperature and is regulated by factors affecting mitochondrial development. *Mol. Microbiol.* 31, 373–379. doi: 10.1046/j.1365-2958.1999.01181.x
- Jiang, F., Rizavi, H. S., and Greenberg, M. L. (1997). Cardiolipin is not essential for the growth of *Saccharomyces cerevisiae* on fermentable or non-fermentable carbon sources. *Mol. Microbiol.* 26, 481–491. doi: 10.1046/j.1365-2958.1997.5841950.x
- Kadenbach, B., Mende, P., Kolbe, H. V., Stipani, I., and Palmieri, F. (1982). The mitochondrial phosphate carrier has an essential requirement for cardiolipin. *FEBS Lett.* 139, 109–112. doi: 10.1016/0014-5793(82)80498-5
- Katayama, K., Sakurai, I., and Wada, H. (2004). Identification of an *Arabidopsis thaliana* gene for cardiolipin synthase located in mitochondria. *FEBS Lett.* 577, 193–198. doi: 10.1016/j.febslet.2004.10.009
- Lewis, R. N., and McElhaney, R. N. (2009). The physicochemical properties of cardiolipin bilayers and cardiolipin-containing lipid membranes. *Biochim. Biophys. Acta* 1788, 2069–2079. doi: 10.1016/j.bbame.2009.03.014
- Li-Beisson, Y., Beisson, F., and Riekhof, W. (2015). Metabolism of acyl-lipids in *Chlamydomonas reinhardtii*. *Plant J.* 82, 504–522. doi: 10.1111/tpj.12787
- Li-Beisson, Y., Shorrosh, B., Beisson, F., Andersson, M. X., Arondel, V., Bates, P. D., et al. (2013). Acyl-lipid metabolism. *Arabidopsis Book* 11:e0161. doi: 10.1199/tab.0161
- Muller, F., and Frentzen, M. (2001). Phosphatidylglycerophosphate synthases from *Arabidopsis thaliana*. *FEBS Lett.* 509, 298–302. doi: 10.1016/S0014-5793(01)03163-5
- Nakamura, Y., Arimitsu, H., Yamaryo, Y., Awai, K., Masuda, T., Shimada, H., et al. (2003). Digalactosyldiacylglycerol is a major glycolipid in floral organs of *Petunia hybrida*. *Lipids* 38, 1107–1112. doi: 10.1007/s11745-006-1166-x
- Osman, C., Haag, M., Wieland, F. T., Brugger, B., and Langer, T. (2010). A mitochondrial phosphatase required for cardiolipin biosynthesis: the PGP phosphatase Gep4. *EMBO J.* 29, 1976–1987. doi: 10.1038/emboj.2010.98
- Pineau, B., Bourge, M., Marion, J., Mauve, C., Gilard, F., Maneta-Peyret, L., et al. (2013). The importance of cardiolipin synthase for mitochondrial ultrastructure, respiratory function, plant development, and stress responses in *Arabidopsis*. *Plant Cell* 25, 4195–4208. doi: 10.1105/tpc.113.118018
- Sato, N., Hagio, M., Wada, H., and Tsuzuki, M. (2000). Requirement of phosphatidylglycerol for photosynthetic function in thylakoid membranes. *Proc. Natl. Acad. Sci. U.S.A.* 97, 10655–10660. doi: 10.1073/pnas.97.19.10655
- Satoh, S., Ikeuchi, M., Mimuro, M., and Tanaka, A. (2001). Chlorophyll b expressed in Cyanobacteria functions as a light-harvesting antenna in photosystem I through flexibility of the proteins. *J. Biol. Chem.* 276, 4293–4297. doi: 10.1074/jbc.M008238200
- Sciara, G., Clarke, O. B., Tomasek, D., Kloss, B., Tabuso, S., Byfield, R., et al. (2014). Structural basis for catalysis in a CDP-alcohol phosphotransferase. *Nat. Commun.* 5:4068. doi: 10.1038/ncomms5068
- Tuller, G., Hrastnik, C., Achleitner, G., Schiefthaler, U., Klein, F., and Daum, G. (1998). YDL142c encodes cardiolipin synthase (Cls1p) and is non-essential for aerobic growth of *Saccharomyces cerevisiae*. *FEBS Lett.* 421, 15–18. doi: 10.1016/S0014-5793(97)01525-1
- Urzica, E. I., Vieler, A., Hong-Hermesdorf, A., Page, M. D., Casero, D., Gallaher, S. D., et al. (2013). Remodeling of membrane lipids in iron-starved *Chlamydomonas*. *J. Biol. Chem.* 288, 30246–30258. doi: 10.1074/jbc.M113.490425
- Wakao, S., Chin, B. L., Ledford, H. K., Dent, R. M., Casero, D., Pellegrini, M., et al. (2014). Phosphoprotein SAK1 is a regulator of acclimation to singlet oxygen in *Chlamydomonas reinhardtii*. *Elife* 3:e02286. doi: 10.7554/eLife.02286
- Wu, F., Yang, Z., and Kuang, T. (2006). Impaired photosynthesis in phosphatidylglycerol-deficient mutant of cyanobacterium *Anabaena* sp. PCC7120 with a disrupted gene encoding a putative phosphatidylglycerophosphate. *Plant Physiol.* 141, 1274–1283. doi: 10.1104/pp.106.083451
- Xu, C., Hartel, H., Wada, H., Hagio, M., Yu, B., Eakin, C., et al. (2002). The *pgp1* mutant locus of *Arabidopsis* encodes a phosphatidylglycerol phosphate synthase with impaired activity. *Plant Physiol.* 129, 594–604. doi: 10.1104/pp.002725
- Yu, B., and Benning, C. (2003). Anionic lipids are required for chloroplast structure and function in *Arabidopsis*. *Plant J.* 36, 762–770. doi: 10.1046/j.1365-3113X.2003.01918.x
- Zhang, M., Su, X., Mileykovskaya, E., Amoscato, A. A., and Dowhan, W. (2003). Cardiolipin is not required to maintain mitochondrial DNA stability or cell viability for *Saccharomyces cerevisiae* grown at elevated temperatures. *J. Biol. Chem.* 278, 35204–35210. doi: 10.1074/jbc.M306729200
- Zhou, Y., Peisker, H., Weth, A., Baumgartner, W., Dormann, P., and Frentzen, M. (2013). Extrplastidial cytidinediphosphate diacylglycerol synthase activity is required for vegetative development in *Arabidopsis thaliana*. *Plant J.* 75, 867–879. doi: 10.1111/tpj.12248

**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2016 Hung, Kobayashi, Wada and Nakamura. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) or licensor are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.