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Vipp1 Is Essential for the Biogenesis of Photosystem I but Not Thylakoid Membranes in *Synechococcus* sp. PCC 7002*[S]

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Background: Vipp1 was previously thought to be essential for viability and biogenesis of thylakoid membranes.
Results: A *vipp1* null mutant of cyanobacterium *Synechococcus* sp. PCC 7002 is viable and assembles thylakoid membranes but lacks Photosystem I.
Conclusion: Vipp1 is not essential but is required for biogenesis of Photosystem I.
Significance: Normal thylakoid biogenesis and structure requires Photosystem I but not Vipp1.

The biogenesis of thylakoid membranes in cyanobacteria is presently not well understood, but the *vipp1* gene product has been suggested to play an important role in this process. Previous studies in *Synechocystis* sp. PCC 6803 reported that *vipp1* (sll0617) was essential. By constructing a fully segregated null mutant in *vipp1* (SynPCC7002_A0294) in *Synechococcus* sp. PCC 7002, we show that Vipp1 is not essential. Spectroscopic studies revealed that Photosystem I (PS I) was below detection limits in the *vipp1* mutant, but Photosystem II (PS II) was still assembled and was active. Thylakoid membranes were still observed in *vipp1* mutant cells and resembled those in a *psaAB* mutant that completely lacks PS I. When the *vipp1* mutation was complemented with the orthologous *vipp1* gene from *Synechocystis* sp. PCC 6803 that was expressed from the strong P_{cpcBA} promoter, PS I content and activities were restored to normal levels, and cells again produced thylakoids that were indistinguishable from those of wild type. Transcription profiling showed that *psaAB* transcripts were lower in abundance in the *vipp1* mutant. However, when the *yfp* gene was expressed from the P_{psaAB} promoter in the presence and the absence of Vipp1, no difference in YFP expression was observed, which shows that Vipp1 is not a transcription factor for the *psaAB* genes. This study shows that thylakoids are still produced in the absence of Vipp1 and that normal thylakoid biogenesis in *Synechococcus* sp. PCC 7002 requires expression and biogenesis of PS I, which in turn requires Vipp1.

Cyanobacteria are considered to be the first oxygen-evolving photolithoautotrophs on Earth. They may have evolved as early as 3.5 billion years ago and are thought to be responsible for the oxygenation of the atmosphere, which began ~2.5 billion years ago (1). In cyanobacteria, photosynthesis antenna pigments capture solar energy, which is subsequently transformed into chemical energy using two reaction centers, Photosystem (PS)[2] I and PS II, and an electron transport chain that connects them (2). Water is oxidized to O_2 during this process, and atmospheric CO_2 is reduced to cellular biomass and carbon storage compounds, such as glycogen, for longer term energy conservation. In cyanobacteria and in algal and plant chloroplasts, the photosynthetic electron transport chain is localized on intracytoplasmic membranes that form the thylakoid membrane network.

Targeting of proteins into and across the thylakoid membranes has been studied and is believed to occur through several pathways, but little is known about the origin of the thylakoid membrane system or how lipids are synthesized, transported, and inserted into this membrane system (3). Moreover, the relationship between the cytoplasmic and thylakoid membranes in cyanobacteria is still very poorly understood. Some researchers propose that these systems are interconnected, whereas others maintain that they are not, and there are arguments in favor of both viewpoints. There is general agreement that the biogenesis of thylakoid membranes is a complex, multidimensional process. During this process, lipids, proteins, and pigments, as well as other cofactors, must be synthesized, transported, assembled, and inserted into these membranes, but few mechanistic details are available.

Several genetic studies have implicated the product of the *vipp1* (vesicle-inducing protein in plastids 1) gene as participating in the process of thylakoid biogenesis (4, 5). Vipp1 was first described as a chloroplast-localized protein in *Pisum sativum*, and further analyses showed that Vipp1 was located on both the inner envelope membrane and the thylakoids (5). This unique localization of Vipp1 on these two membranes led to the presumption that Vipp1 might be involved in the assembly of the thylakoid membrane system (5). This presumption was supported by the characterization of a mutant of *Arabidopsis thaliana*, in which the expression of the *vipp1* gene was strongly

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[2] The abbreviations used are: PS, Photosystem; Chl, chlorophyll; PBP, phycobiliproteins; PG, phosphatidylglycerol; Comp, *trans*-complemented *vipp1* mutant strain.

reduced (4). However, a fully segregated null mutant for *vipp1* could not be produced, and thus the product of *vipp1* was believed to be essential for viability (4). The mutant plants expressed only about 20% of the Vipp1 protein levels that occur in wild type under normal growth conditions, and these knockdown mutants were incapable of photoautotrophic growth on soil (4).

Genes similar to *vipp1* are also found in most cyanobacteria (6–8). Recently, attempts have been made to construct *vipp1* mutants of *Synechocystis* sp. PCC 6803, but although the level of Vipp1 could be lowered, in none of these studies could null mutations of the *vipp1* gene be fully segregated. These *Synechocystis* sp. PCC 6803 merodiploids had a phenotype similar to that of the knockdown strains of *A. thaliana*, and they exhibited a comparable loss of thylakoid membrane content and structure and also had reduced photosynthetic activity (6, 9). Thus, it was suggested that Vipp1 is also essential in cyanobacteria, because it apparently plays an essential role in thylakoid membrane biogenesis. However, because null alleles of *vipp1* never fully segregated in these *Synechocystis* sp. PCC 6803 strains, the results obtained from the characterization of the merodiploid strains were inconclusive and must be interpreted cautiously.

By using an indirect route for the construction of a *vipp1* mutant in the cyanobacterium *Synechococcus* sp. PCC 7002, we show here that a *vipp1* null mutant can be constructed and that the fully segregated null mutant is viable. This mutant strain could not grow photoautotrophically, but it could be grown photoheterotrophically when supplied with glycerol under very low irradiance conditions. When this *vipp1* mutant was complemented with the *vipp1* gene from *Synechocystis* sp. PCC 6803 expressed from the strong P_{cpcBA} promoter, the resulting strain regained the ability to grow photoautotrophically and regained all other phenotypic properties of the wild type. Characterization of these strains showed that Vipp1 is required for biogenesis of PS I and that PS I is required for the biogenesis of "normal" thylakoid membranes in *Synechococcus* sp. PCC 7002.

EXPERIMENTAL PROCEDURES

Strains, Culture Conditions, and Transformation Procedure—The wild-type strain of *Synechococcus* sp. PCC 7002 and the *vipp1* mutant strain complemented with the *vipp1* gene from *Synechocystis* sp. PCC 6803 (see below) were grown in liquid A^+ medium under standard conditions (10): at 38 °C with an irradiance of 250 μmol photons m^{-2} s^{-1} provided by cool white fluorescent lights and with sparging with 1% (v/v) CO_2 in air. Mutant strains were grown under low irradiance conditions (~10 μmol photons m^{-2} s^{-1}), and the A^+ medium was supplemented with 20 mM glycerol, which served as the main carbon and energy source. For mutant strains, appropriate antibiotics were added as required at the following concentrations: spectinomycin (50 μg/ml); gentamycin (20 μg/ml); kanamycin (100 μg/ml); and erythromycin (20 μg/ml). Transformation of *Synechococcus* sp. PCC 7002 was performed as described previously (11).

Generation of vipp1 Deletion Mutant and a trans-Complemented Strain—In agreement with the results of others and despite many attempts, direct deletion of *vipp1* was never successful. Thus, a different strategy was employed. First, a PS I-less strain of *Synechococcus* sp. PCC 7002 was constructed by deleting the *psaAB* genes (12). The *vipp1* gene in this strain was then inactivated by a homologous recombination strategy by deleting a part of the gene (bp 287–373 of the coding sequence); the deleted region was replaced with a DNA fragment encoding *aacC1*, which confers resistance to gentamycin (Fig. 1*A*). The PCR primers used to amplify the flanking sequence regions for this approach are given in Table 1. The Δ*psaAB*::*aadA* mutant strain was rescued at the *psaAB* locus by transformation with plasmid pAQEEmr80 as described previously (13) to produce a $PsaAB^+$ $Vipp1^-$ (*vipp1*::*aacC1*) mutant strain. Full segregation of the wild-type and *vipp1*::*aacC1* alleles was verified by PCR analyses and DNA sequencing of amplicons derived from the DNA of the *vipp1*::*aacC1* null mutant strain.

For the construction of a strain in which the *vipp1*::*aacC1* mutation was complemented in *trans*, the orthologous *vipp1* gene (open reading frame sll0617) from *Synechocystis* sp. PCC 6803 was amplified by PCR using Phusion DNA polymerase (New England Biolabs) and was introduced into plasmid pAQ1Ex-*PcpcBA* using primer set ExR and ExF (Table 1) as described previously (14). The resulting plasmid, pAQ1Ex-*PcpcBA*::sll0617, was transformed into the *vipp1*::*aacC1* mutant to generate a strain in which the *vipp1* null mutation was heterologously complemented by expression of the sll0617 product from the strong *cpcBA* (phycocyanin) promoter (P_{cpcBA}; also derived from *Synechocystis* sp. PCC 6803; see Ref.

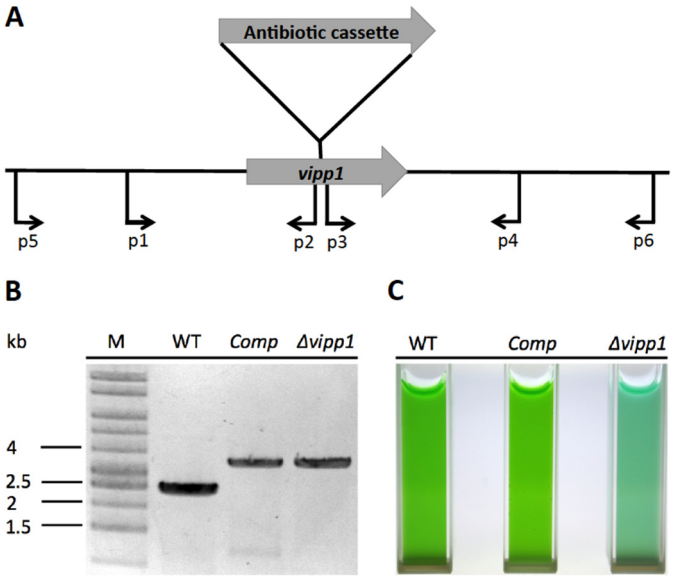

FIGURE 1. **Construction and verification of *vipp1* mutant as well as a *trans*-complemented strain.** *A*, scheme showing the construction of a *vipp1* mutant by homologous recombination by using primer set p1 and p2 to amplify the upstream region and primer set p3 and p4 to amplify the downstream region. An antibiotic resistance cassette was ligated into restriction sites added to the appropriate ends of the flanking sequences (see "Experimental Procedures" for other details). *B*, results of agarose gel electrophoresis of amplicons produced using primers p5 and p6, showing the fully segregated interruption mutant of *vipp1*. The template DNAs were isolated from the wild type (*WT*), the *trans*-complemented *vipp1* mutant (*Comp*), and the *vipp1* mutant strain (Δ*vipp1*). The results clearly showed that the wild-type *vipp1* and interrupted *vipp1*::*aacC1* alleles had segregated completely in the *vipp1* mutant and that the *vipp1* gene was still mutated in the Comp strain. *Lane M*, DNA size markers. *C*, cultures of equivalent cell density ($OD_{730\,nm}$ = 1.0) for WT, Comp, and Δ*vipp1*.

14). The resulting complemented mutant strain (*vipp1*::*aacC1* pAQ1Ex-*PcpcBA*::sll0617) was repeatedly streaked and grown photoautotrophically in A^+ medium under standard irradiance conditions (*i.e.* 250 μmol of photons m^{-2} s^{-1}).

Pigment Analysis—Chlorophyll (Chl) *a*, carotenoid, and phycobiliprotein (PBP) concentrations were measured as described (15). Pigment concentrations were compared on the basis of equal cell numbers, which were determined from the optical density at 730 nm ($OD_{730\ nm}$; 1.0 $OD_{730\ nm}$ = 1.0 ± 0.2 × 10^8 cells ml^{-1}; see Ref. 15). These measurements were made with cells that had been harvested by centrifugation from cultures grown to late exponential growth phase ($OD_{730\ nm}$ = ~0.6 to 0.7 ml^{-1}) and resuspended in 50 mM Tris-HCl, pH 7.0 buffer. Chl *a* and carotenoids were extracted from cells with 100% methanol, and their concentrations were determined as described (15). To determine relative PBP levels, cells were incubated at 65 °C for 8 min, and a difference spectrum with untreated control cells was recorded as described previously (15).

Polyacrylamide Gel Electrophoresis and Immunoblotting—Polyacrylamide gel electrophoresis (PAGE) in the presence of sodium dodecyl sulfate (SDS) was performed as described (12) on 15% (w/v) polyacrylamide slab gels (30.0:0.8 acrylamide/bisacrylamide). Equal amounts of cells (100 μl of $OD_{730\ nm}$ = 4) were centrifuged, and the cell pellets were collected and resuspended in 40 μl of BugBuster™ protein extraction reagent (Novagen, Madison, WI). Cells were disrupted after 20 min of incubation at room temperature. Aliquots (20 μl) of the whole cell extract were loaded to each lane. Rabbit antibodies against PsaA, PsaB, and Vipp1 were purchased from Agrisera (Vännäs, Sweden). Immunoblotting was performed as described previously (13).

Oxygen Evolution Assay—Whole-chain oxygen evolution as well as respiratory oxygen uptake in wild-type and mutant cells were measured using a Clark-type electrode as described (16). The temperature of the measuring chamber was maintained at 38 °C by a circulating water bath. Cells were washed once with fresh A^+ medium and adjusted to equal final cell concentration on the basis of $OD_{730\ nm}$. For oxygen evolution measurements, 10 mM $NaHCO_3$ was added to the cell suspension. Respiration rates were obtained under the same conditions but without illuminating the cells.

Fluorescence Emission Spectra at 77 K—Fluorescence emission spectra at 77 K were measured with an SLM 8000C spectrofluorometer, modified by OLIS, Inc., as described (12). Cells in exponential growth phase ($OD_{730\ nm}$ = ~0.6–0.7 ml^{-1}) were collected and resuspended in 50 mM Tris-HCl, pH 7.0, buffer. Glycerol was added to a final concentration of 60% (v/v). Cells were adjusted to a concentration of ~0.5 $OD_{730\ nm}$ ml^{-1} and quickly frozen in liquid nitrogen. The excitation wavelength for Chl excitation was 440 nm. A long pass filter (transmitting at 600 nm) was used at the inlet of the emission monochromator to minimize contributions from scattered light.

Whole-cell P700 Activity Measurements—Cells (final $OD_{730\ nm}$ = ~0.5) were collected and resuspended in 50 mM Tris-HCl, pH 8.3, buffer. The absorbance change at 700 nm was monitored by a model JTS-10 LED pump-probe spectrometer (Bio-Logic). A high power red LED (680 ± 50 nm) provided the actinic illumination. A high power white LED, filtered through a 700-nm interference filter (Edmund Optics, Inc.), provided the measuring pulses.

Transmission Electron Microscopy—Thylakoid membranes from wild-type and mutant cells of *Synechococcus* sp. PCC 7002 were visualized by transmission electron microscopy of thin sections as described (17). The ultrathin sections were viewed with a JEM-1200 transmission electron microscope (JEOL Ltd.). Images were captured using TIETZ digital image capture software.

Lipid Body Detection—Nile Red staining was performed to detect lipid bodies using a reported method with minor modifications (18). Cells were harvested, washed, and resuspended in 50 mM Tris-HCl, pH 8.0, buffer. Nile Red stock solution (1 μl of a 1 mg ml^{-1} stock solution in dimethyl sulfoxide) was added to an aliquot of washed cells (100 μl). After staining for 10 min, lipid bodies inside cells were visualized by fluorescence using a FluoView FV1000 confocal microscope (Olympus, Center Valley, PA) in scanning mode. The excitation wavelength was 488 nm, and an emission wavelength of 500–600 nm was selected for all experiments.

Total mRNA Profiling—Transcriptome profiling was performed as described (10). The *vipp1* mutant strain and Comp strain were first adapted to low irradiance (~10 μmol photons m^{-2} s^{-1}) on medium A^+ supplemented with glycerol (20 mM). Cells were reinoculated and harvested at $OD_{730\ nm}$ = 0.7. Total RNA was then extracted as described (18). The construction of cDNA libraries and sequencing (SOLiD™) were performed in the Genomic Core Facility at Pennsylvania State University. Mapping against the *Synechococcus* sp. PCC 7002 genome was performed using the BWA software package (19). The resulting alignment files were further analyzed with self-developed scripts to extract expression levels for each gene as described previously (10). The RNA sequencing data were deposited in the NCBI Sequence Read Archive under accession number SRP035555.

Yellow Fluorescent Protein (YFP) Detection—To determine whether Vipp1 plays a direct regulatory role in the transcription of the *psaAB* and *chlLN* operons, promoter regions for *psaAB* and *chlLN* were amplified and transcriptionally fused to *yfp* as well as *aphII* (conferring kanamycin resistance) separately. The primers used to amplify these promoter regions for *psaAB* (psaABF and psaABR) and *chlLN* (chlLNF and chlLNR) are listed in Table 1. The fused constructs were then used to replace open reading frame SYNPCC7002_A2746 (Fig. 2*A*) in both the *vipp1* mutant and the complemented strain by homologous recombination as described (11). Primers used to amplify the upstream and downstream flanking regions of SYNPCC7002_A2746 are listed in Table 1 (A2746upF, A2746upR, A2746downF, and A2746downR). Transcription profiling analyses under many different conditions showed that ORF SYNPCC7002_A2746 produces few if any transcripts under most growth conditions (10, 20, 21). Additionally, a deletion mutant of SYNPCC7002_A2746 has been constructed (Fig. 2*B*). No detectable growth phenotype was observed for this mutant strain compared with wild type strain, and thus this gene site was used as a neutral site (Fig. 2*C*). Full segregation of the *vipp1* mutant strain and the complemented strain containing the promoter fusions to *yfp* was verified using primer set A2746upF and

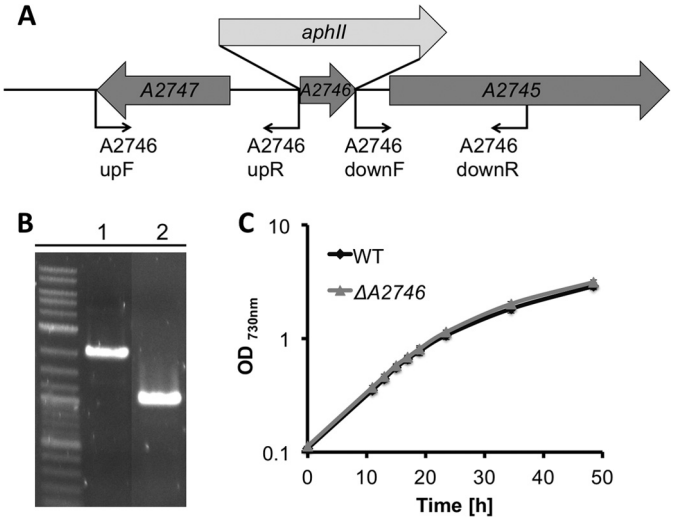

FIGURE 2. **Construction and verification of a neutral site platform for *Synechococcus* sp. PCC 7002.** *A*, diagram showing the construction of a SynPCC7002_A2746 mutant and positions of oligonucleotide primers (see Table 1). *B*, agarose gel electrophoresis of amplicons produced by polymerase chain reaction, demonstrating complete segregation of alleles for SynPCC7002_A2746::*aphII* and SynPCC7002_A2746, using primer set A2746upF and A2746downR. The template DNAs were isolated from the SynPCC7002_A2746 mutant (*lane 1*) and wild type (*lane 2*). *C*, comparison of growth rates for *Synechococcus* sp. PCC 7002 WT and a neutral site mutant strain constructed in open reading frame SynPCC7002_A2746 (Δ*A2746*). The growth rates were indistinguishable within experimental error. The data are the average of three biological replicates.

A2746downR. The products were sequenced to verify that no inadvertent changes had occurred during strain construction. These strains were grown under low irradiance (~10 μmol photons m^{-2} s^{-1}) on medium A^+ supplemented with glycerol (20 mM). Cells were harvested by centrifugation from exponential growth phase ($OD_{730\,nm}$ = ~0.6–0.7 ml^{-1}), resuspended in A^+ medium containing 20 mM glycerol, and adjusted to the same $OD_{730\,nm}$ = 0.5 ml^{-1}. YFP fluorescence in these cells was detected with an SLM 8000C spectrofluorometer, modified by OLIS, Inc. (Bogart, GA). The excitation wavelength was 488 nm, and emission spectra were recorded from 500 to 600 nm.

RESULTS

Generation of vipp1 Mutant—Genetic manipulations to generate a *vipp1* null mutant, as well as a strain (denoted Comp) in which the resulting *vipp1* mutation was complemented in *trans*, are described under "Experimental Procedures." For construction of a *vipp1* null mutant, *Synechococcus* sp. PCC 7002 was first adapted to grow on glycerol as a carbon/energy source, which allows one to construct mutants lacking PS I and/or PS II (12). Some observations made with *vipp1* merodiploid strains suggested that these merodiploid strains had greatly reduced levels of PS I (data not shown, but see below), and thus a strain in which the *psaAB* genes had been deleted was used for inactivation of the *vipp1* gene. After several attempts, a fully segregated *vipp1*::*aacC1* strain, which was verified by PCR analysis as shown in Fig. 1*B*, was obtained. Although the *vipp1* gene was believed to be essential in cyanobacteria (6, 9), the results in Fig. 1*B* show that the *vipp1* product is not essential in *Synechococcus* sp. PCC 7002 and that a null mutant is viable. Because it was much easier to obtain a fully segregated *vipp1* null mutation in a PS I-less strain, it appeared likely that Vipp1 was somehow involved in the biogenesis of PS I.

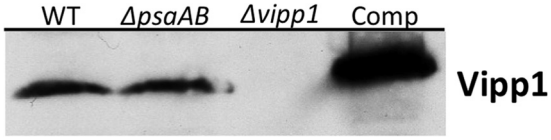

FIGURE 3. **Immunoblotting of whole cell extracts of *Synechococcus* sp. PCC 7002 strains with antibodies to Vipp1.** Antibodies to Vipp1 were used to detect Vipp1 levels in WT, Comp, Δ*vipp1*, and a PS I-less mutant (Δ*psaAB*). Equal quantities of cells were used to produce the extracts for this experiment, so the Vipp1 levels detected in this experiment can be compared semiquantitatively.

Characterization of the Mutant Strain—The *vipp1* mutant strain could not grow photoautotrophically, but this strain could still grow photoheterotrophically under low irradiance conditions (~10 μmol photons m^{-2} s^{-1}) when cells were supplied with 20 mM glycerol. Immunoblotting showed that no Vipp1 protein was detectable in the mutant cells (Fig. 3). Previous studies had shown that a deficiency in Vipp1 led to impairment of the biosynthesis of thylakoid membranes in plants and *Synechocystis* sp. PCC 6803 (4, 9). Transmission electron microscopy of thin section cells was used to examine the membrane organization in the *vipp1* mutant strain. As shown in Fig. 4*B*, cells of the *vipp1* mutant had far fewer thylakoid membranes than the wild type (Fig. 4*A*). However, some vestigial thylakoid membranes were still present, and this indicated that Vipp1 is not essential for the biogenesis of thylakoid membranes. It should be noted, however, that the thylakoid membranes appeared to have a much simpler organization in the *vipp1* mutant and that in some cells, the thylakoid membranes appeared to be directly connected to the cytoplasmic membrane (see Fig. 4*B*).

Complementation of the vipp1 Mutation in Trans—Because of the multistep procedure employed to construct the *vipp1* mutant strain, it was important to demonstrate that the resulting *vipp1* mutant could be complemented to rescue a wild-type phenotype. To avoid potential problems arising from homologous recombination of *vipp1* alleles, the orthologous *vipp1* gene (locus tag, sll0617) from *Synechocystis* sp. PCC 6803 was used for complementation instead of *vipp1* from *Synechococcus* sp. PCC 7002. Vipp1 from *Synechocystis* sp. PCC 6803 is 54% identical and 71% similar in sequence to Vipp1 from *Synechococcus* sp. PCC 7002. A plasmid was constructed in which ORF sll0617 from *Synechocystis* sp. PCC 6803 was placed under the control of the strong P_{cpcBA} promoter (also from *Synechocystis* sp. PCC 6803 (14)), and this plasmid was transformed into the *vipp1* null mutant. As shown in Fig. 1*B*, PCR analysis using primer set p5 and p6 (Table 1) showed that the *vipp1* gene was still interrupted in the resulting complemented strain, which was denoted as strain "Comp." Immunoblotting (Fig. 3) showed that the complemented mutant strain accumulated much more Vipp1 protein than the wild type. The complemented strain could grow photoautotrophically (Fig. 1*C*) and was no longer sensitive to high light conditions. Analysis of thin sections by transmission electron microscopy showed that the thylakoid membranes of the Comp strain cells were indistinguishable from those in wild-type cells (Fig. 4*C*). These results demonstrate that *vipp1* (sll0617) from *Synechocystis* sp. PCC 6803 can

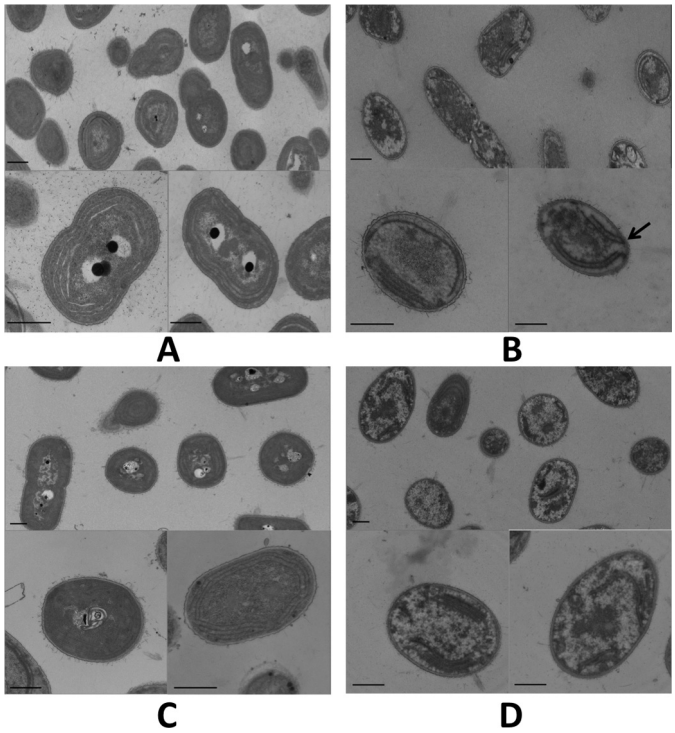

FIGURE 4. **Thylakoid membrane morphology as revealed by electron microscopy.** Thin sections of *Synechococcus* sp. PCC 7002 cells from various strains were examined by transmission electron microscopy. *A*, thylakoid membranes were normally assembled in WT (*A*) and in Comp (*C*). Thylakoids were greatly reduced in number and area in the *vipp1* mutant (*B*), and these cells closely resembled cells for a *psaAB* mutant (*D*). The *arrows* in the *bottom right* of *B* show thylakoids that clearly appear to be directly connected to the cytoplasmic membrane. *Bars*, 500 nm.

TABLE 1
Oligonucleotide primers used in this study

Name	Sequence (5′–3′)
p1	CATTGACTACCGAATACACCAAGGTGCAA
p2	GGTGTCCGCAAAGCTTTTTTTCCGGACTAG
p3	GAAAACCTCCTTGCTCTAGAAAGCAAAATC
p4	AGACAGTTCCTTACGTTAACAGTTTTCGG
p5	GCTAACTCGGCATCATTGGTATTCGTCAGC
p6	TCAGTATCGGGATCAACGGCAACCCCATAA
ExF	GGTCGTCATATGGGATTATTTGACCGTTTA
ExR	GCAGCCGGATCCTTACAGATTATTTAACCG
psaABF	ATTCTGCCATGGCCTTTTCGGGTTAAACCT
psaABR	GACTGCCATATGAGGATTCTCCTCTCTAGA
chlLNF	CCTTTTGAATTCTTCAACATGACGGCAACG
chlLNR	TCATTGCCATGGGATTGCGTGCTCCTAAAA
A2746upF	TGCGACTCGTTTTGACATTCCGCCAAAACA
A2746upR	ACCATTGTCGACACCAGAGGAGACTTTAAA
A2746downF	CTTCGGGAATTCAGCAACGTTTTGAGGGAA
A2746downR	CCCCGCAAAATTTCCTTCGCGCCGCTTGTA

fully complement the *vipp1* mutant of *Synechococcus* sp. PCC 7002 and could restore a wild-type phenotype (see additional results, and see "Discussion").

Pigments and Oxygen Evolution Rate—The *vipp1* mutant was noticeably bluer in color than the wild type (Fig. 1*C*), so we next analyzed the pigment content and oxygen evolution behavior of the *vipp1* mutant and the Comp strain (Table 2). The Chl *a* content of the *vipp1* mutant was reduced to about 10% and the total carotenoid content was reduced to about 20% of the levels in the wild type. The PBP content of the *vipp1* mutant was approximately half that of the wild type (Table 2). The increased PBP content relative to the Chl *a* explains the bluish color of the cells. These values returned to nearly wild-type levels when the *vipp1* mutation was complemented in *trans*. Interestingly, the Chl *a* and carotenoid levels in the *vipp1* mutant were similar to those in a PS I-less mutant, although the PBP content of the PS I-less mutant was essentially the same as that of the wild type (Table 2).

Oxygen evolution rates and respiratory oxygen uptake rates were measured for these strains (Fig. 5). The measurements were either compared on the basis of equal cell number, as determined by the optical density at 730 nm (Fig. 5*A*), or on the basis of the Chl *a* content (Fig. 5*B*). As shown in Fig. 5*A*, the *vipp1* mutant evolved oxygen, but this activity rapidly declined after several min of illumination (data not shown). The initial oxygen evolution rate on the basis of Chl *a* was roughly 2.5-fold higher than that of the wild-type strain but was only about 25% of the wild-type rate on a per cell basis. These behaviors are very similar to those of a strain lacking PS I (12). When the *vipp1* mutation was complemented in *trans*, the oxygen evolution and respiratory uptake rates for the resulting Comp strain were very similar to those of the wild type. Thus, PS II complexes in the *vipp1* mutant and the PS I-less strain were assembled and were functional. The similar pigment contents and oxygen evolution behaviors of these two strains further suggested that the *vipp1* mutant probably had fewer PS I complexes per cell.

Low Temperature Fluorescence Emission Spectroscopy—Fig. 6 shows the low temperature (77 K) fluorescence emission spectra of various *Synechococcus* sp. PCC 7002 strains. When the excitation wavelength was 440 nm to excite Chl *a*, three major emission peaks were observed at 685, 695, and 715 nm for the wild type. The first two peaks principally arise from PS II, whereas the emission peak at 715 nm arises from PS I (13). The fluorescence emission spectrum of the *vipp1* mutant shows no emission peak from PS I and only shows emission peaks associated with PS II. The emission spectrum for the *vipp1* mutant after *trans*-complementation with sll0617 from *Synechocystis* sp. PCC 6803 was nearly indistinguishable from that of the wild type (Fig. 6). These data strongly implicate Vipp1 in the expression or biogenesis of PS I.

PS I Activities in Whole Cells—The low temperature fluorescence emission spectrum of the *vipp1* mutant showed that the PS I content of this strain was severely reduced. To verify that the PS I activity was similarly reduced in the *vipp1* mutant, photobleaching of P700 was directly measured at 700 nm in whole cells with a pump-probe spectrophotometer as described under "Experimental Procedures." As shown in Fig. 7, photobleaching of P700 occurred when whole cells were illuminated with actinic light, and a slight increase in photobleaching occurred over a 10-s period of illumination. The absorption change at 700 nm was fully reversible when the actinic light was switched off. No photobleaching at 700 nm was detectable for the *vipp1* mutant, but the photobleaching of P700 in the *trans*-complemented strain was similar in magnitude and kinetics to that of the wild type. PS I complexes were isolated from the wild type and from the *trans*-complemented Comp strain. Time-resolved optical spectroscopy on the millisecond time scale showed that the PS I complexes from these two strains had similar extents of photobleaching and lifetimes of charge separation (data not shown). These spectroscopic studies show that

TABLE 2

Pigment contents of *Synechococcus* sp. PCC 7002 WT, Comp, Δ*psaAB*, and Δ*vipp1*

The data shown are averages and S.D. values for three biological replicates.

	WT	Comp	Δ*psaAB*	Δ*vipp1*
Chlorophyll *a* (μg ml^{-1} OD$_{730\ nm}$$^{-1}$)	7.15 ± 0.16	7.11 ± 0.22	0.88 ± 0.02	0.71 ± 0.06
Carotenoids (μg ml^{-1} OD$_{730\ nm}$$^{-1}$)	2.25 ± 0.05	2.62 ± 0.07	0.68 ± 0.01	0.41 ± 0.04
Phycobiliproteins (relative amount)	372 ± 4	306 ± 8	365 ± 3	202 ± 2

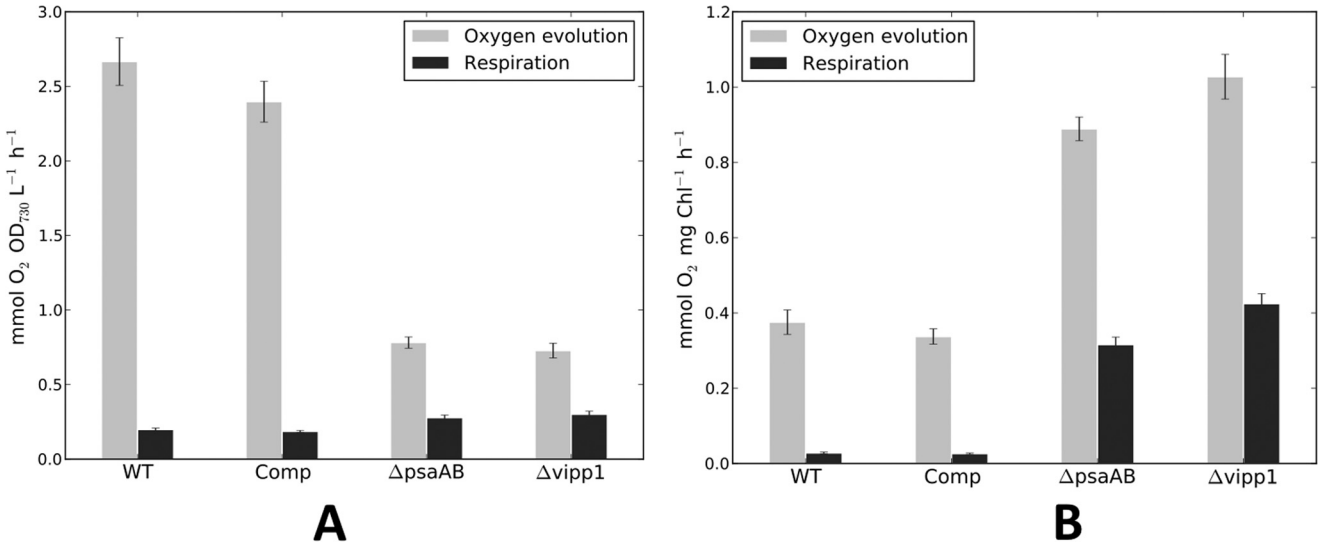

FIGURE 5. **Oxygen evolution and respiration rates for WT, Comp, Δ*psaAB*, and Δ*vipp1*, based on equal cell numbers (based on OD$_{730\ nm}$) (*A*) or equal Chl *a* (*B*).** The *vipp1* mutant strain as well as the *psaAB* mutant strain had much higher oxygen evolution rates than the WT strain when rates were compared on the basis of Chl but much lower oxygen evolution rates when rates were compared on the basis of equal cell numbers. Note that these values were derived from the initial rates of oxygen evolution for the *vipp1* and the *psaAB* mutant strains, because oxygen evolution rates rapidly declined for these two strains that had no PS I activity to drive the reoxidation of the plastoquinone pool. The data shown are averages values for three biological replicates, and the *error bars* show the standard deviation.

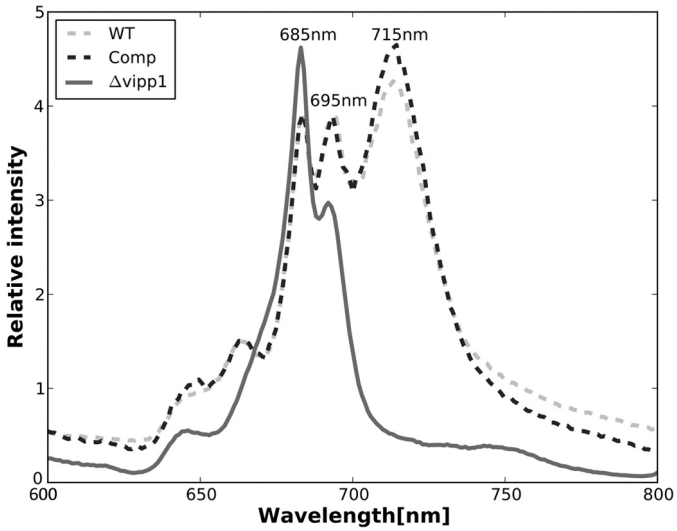

FIGURE 6. **Low temperature (77 K) fluorescence emission spectra of whole cells of WT, Comp, and Δ*vipp1*.** In the Δ*vipp1* mutant, PS I fluorescence emission at ~715 nm was completely absent, but PS II was still assembled and exhibited normal fluorescence emission at 685 and 695 nm. The excitation wavelength was 440 nm.

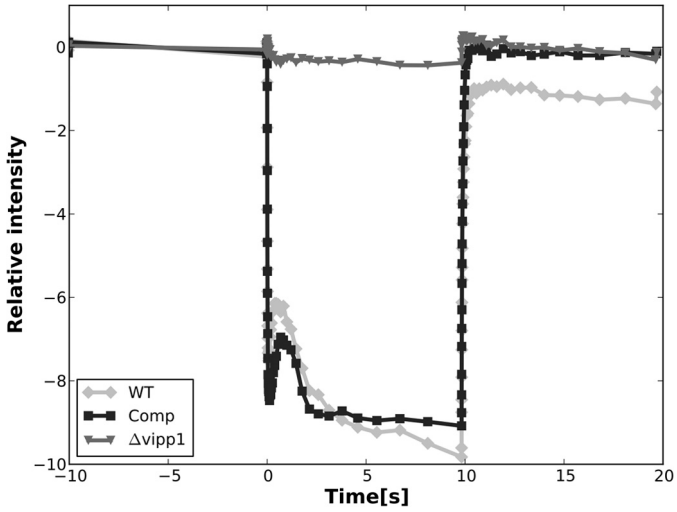

FIGURE 7. **Photobleaching of P700 in whole cells of WT (*light gray line*), Comp (*black line*), and Δ*vipp1* (*dark gray line*).** The PS I activity in the *trans*-complemented *vipp1* mutant strain was almost the same as that of the WT. No P700 photobleaching activity was detected in the *vipp1* mutant strain. The actinic light was turned on at 0 s and turned off after 10 s, and absorption difference was measured at 700 nm.

the *vipp1* mutant does not assemble functional PS I complexes. Heterologous complementation of *vipp1* with ORF sll0617 from *Synechocystis* sp. PCC 6803 was sufficient to restore normal PS I biogenesis and functionality to the *vipp1* mutant of *Synechococcus* sp. PCC 7002.

Immunoblotting to Detect PS I Polypeptides—Whole-cell extracts of the wild type, the *vipp1* mutant strain, and the *trans*-complemented *vipp1* mutant strain were prepared and subjected to SDS-PAGE, and the resolved proteins were transferred to membrane filters for immunoblotting. Previous

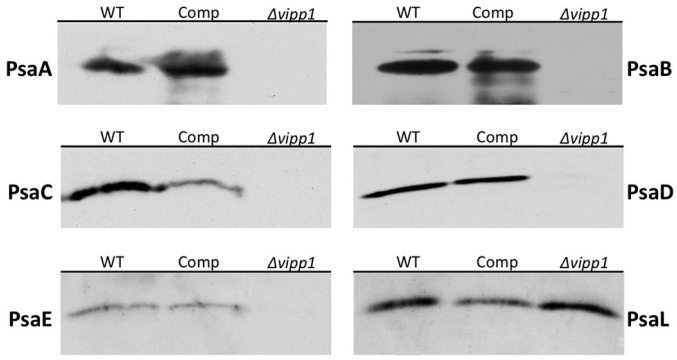

FIGURE 8. **Immunoblotting of PS I subunits.** Antibodies to PsaA, PsaB, PsaC, PsaD, PsaE, and PsaL were used to detect the presence of the PS I subunits in whole cell extracts of WT, Comp, and Δ*vipp1*. PsaL was the only PS I subunit detected in the *vipp1* mutant strain, but all subunits were detected in the WT control and Comp strains. Note that these immunoblots were performed with whole cell extracts of cells scraped from plates; thus, the results are only qualitative and should not be compared quantitatively.

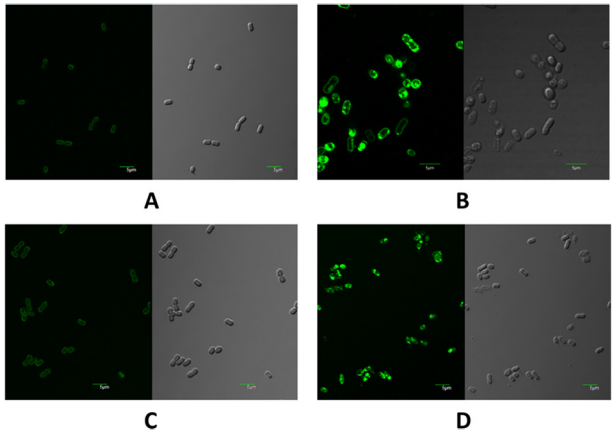

FIGURE 9. **Confocal fluorescence microscopy images (*left*) and differential interference contrast images (*right*) of *Synechococcus* sp. PCC 7002 cells stained with Nile red.** For the fluorescence images, the excitation wavelength was 488 nm, and emitted light in the wavelength range from 500 to 600 nm was detected. *Scale bars*, 5 μm. *A*, cells of wild-type *Synechococcus* sp. PCC 7002. *B*, cells of the *vipp1* mutant strain. *C*, *trans*-complemented cells of the *vipp1* mutant strain. *D*, cells of the *psaAB* mutant strain.

studies have shown that PsaC, PsaD, and PsaE, which are water-soluble and form the stromal ridge of PS I (2, 12), do not accumulate in PS I-less mutants (12). Immunoblotting showed that PsaA, PsaB, PsaC, PsaD, and PsaE do not accumulate in the *vipp1* mutant, which is consistent with all other evidence indicating that PS I is missing in the *vipp1* mutant (Fig. 8). However, all of these proteins were detected in the wild type and in the Comp strain. Interestingly, the integral membrane protein, PsaL, which is responsible for trimerization of PS I (22), accumulated in the absence of PsaA and PsaB in the *vipp1* mutant (Fig. 8). This observation is consistent with observations made for *Synechocystis* sp. PCC 6803, for which it was also found that PsaL accumulated in membranes independently of the levels of PsaA and PsaB (23).

Thylakoid Membranes in a psaAB Deletion Mutant—Although previous studies had suggested that Vipp1 was required for thylakoid membrane biogenesis, the results shown above suggested that Vipp1 is required for *psaAB* expression or the biogenesis of PS I. The presence of vestigial thylakoid membranes in the *vipp1* mutant indicated that normal thylakoid biogenesis might require PS I assembly rather than Vipp1. A natural hypothesis arising from these observations is that a PS I-less mutant should produce vestigial thylakoids that closely resemble those in the *vipp1* mutant. Fig. 4*D* shows images of cells of a *psaAB* deletion mutant of *Synechococcus* sp. PCC 7002 that is unable to assemble any functional PS I complexes (or subcomplexes (12)). Immunoblotting showed that the PS I-less mutant cells accumulated wild-type levels of Vipp1 (Fig. 3). Like the *vipp1* mutant, cells of the *psaAB* deletion mutant produce vestigial thylakoids that closely resemble those of the *vipp1* mutant. Therefore, these results clearly demonstrate that the capacity to produce wild-type thylakoid membranes requires the normal biogenesis of PS I rather than Vipp1.

Detection of Lipid Bodies—Previous studies in plant chloroplasts and *Synechocystis* sp. PCC 6803 suggested that *vipp1* might play a role in membrane biogenesis and might more specifically affect the insertion of membrane lipids (24). However, the results presented here conclusively demonstrate that cyanobacterial Vipp1 plays a role in the expression or biogenesis of PS I. Nile red is a lipid-soluble fluorescent dye that can be used to detect lipids in microorganisms (18). To determine whether lipid bodies were produced in the strains constructed here, Nile red staining was performed to detect lipid bodies. The *vipp1* mutant accumulated numerous lipid bodies in the cytoplasm (Fig. 9*B*), but lipid bodies were not observed in wild type (Fig. 9*A*). This result excludes the possibility that Vipp1 is involved in the biogenesis of lipid bodies, but it suggests that Vipp1 is directly or indirectly involved in the assembly of lipids into thylakoid membranes. The inability to produce normal amounts of PS I apparently interferes with lipid insertion into the thylakoid membranes, and the lipids apparently then accumulate as lipid bodies in the cytoplasm. Consistent with the results described above, the PS I-less strain also accumulated lipid bodies in the cytoplasm that resembled those in the *vipp1* strain (Fig. 9*D*). When the ability to produce PS I was restored by *trans*-complementation of the *vipp1* mutation, lipids no longer accumulated as lipid bodies in the cytoplasm of the Comp strain (Fig. 9*C*).

Vipp1 Is Not Required for Transcription of psaAB—In the experiments described above, we showed that PS I is not detectable in cells that lack Vipp1 and that restoration of Vipp1 by *trans*-complementation reverses all known phenotypic defects associated with the absence of Vipp1. Fig. 10 shows a scatter plot that compares the transcript abundances for each gene in the *vipp1* deletion strain in comparison with their abundances in the Comp strain. Four genes, *psaAB* and *chlLN*, which occur in two dicistronic operons, showed significantly lower transcript abundances in the *vipp1* mutant compared with the *trans*-complemented strain. These data suggested that Vipp1 might regulate *psaAB* transcript levels.

To ascertain whether Vipp1 was acting as an activator or repressor of transcription of the *psaAB* operon, we introduced a promoter fusion of P_{psaAB} to *yfp* (P_{psaAB}::*yfp*) into a neutral site (SYNPCC7002_A2746) in the *Synechococcus* sp. PCC 7002 chromosome in the *vipp1* mutant as well as the *trans*-complemented Comp strain (Fig. 11, *A* and *B*). The Yfp fluorescence in these two strains was equal within error (Fig. 11*C*). Similar results were observed for the P_{chlLN} fused to (P_{chlLN}::*yfp;* data

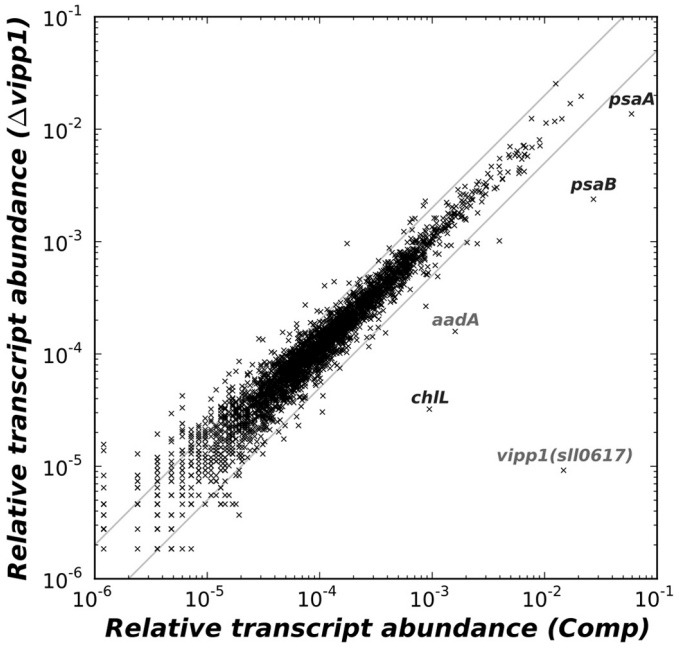

FIGURE 10. **Scatter plot comparing the relative transcript abundances for mRNAs of the *vipp1* mutant to those in the *trans*-complemented *vipp1* mutant strain.** Transcripts for *psaA* and *psaB* were specifically higher in the *trans*-complemented *vipp1* strain, which also had enhanced mRNA levels for the *vipp1* (sll0617) gene from *Synechocystis* sp. PCC 6803. Transcript levels for *chlL* were also more abundant in the *trans*-complemented strain, but nearly all other transcripts were unchanged. The *gray lines* indicate 2-fold changes in transcript levels.

not shown). These results show that Vipp1 is not directly involved in regulating the transcription of *psaAB*. However, it is possible that Vipp1 plays a role in PS I biogenesis that indirectly influences the stability of the *psaAB* mRNA (*e.g.* by influencing translation of this transcript). The presence or absence of Vipp1 in cells did not cause a change in the amount of YFP expression. Because the *vipp1* mutant strain can only be grown at very low irradiance levels, it is possible that the ChlLN operon, which encodes two of the three subunits of the light-independent protochlorophyllide reductase (25), is specifically transcribed at higher levels in the Comp strain. This strain, which is derived from the *vipp1* mutant, has a much greater demand for Chl *a* than the parental strain because of restoration of PS I. Low irradiance growth conditions might cause a limitation for Chl *a* biosynthesis at the level of protochlorophyllide *a* reduction, which typically is catalyzed by the light-dependent protochlorophyllide *a* reductase (26). The low light levels might lead to derepression of the light-independent protochlorophyllide *a* reductase, ChlNBL, in the Comp strain because of an increased demand for Chl *a* to assemble PS I. This would not occur in the *vipp1* mutant because it does not assemble PS I and requires only 10% of the Chl found in the wild type (Table 2).

DISCUSSION

The exact role of Vipp1 has been a longstanding mystery that has been investigated in several organisms over about 2 decades. However, its function is still unclear. A *vipp1* merodiploid knockdown strain of *Synechocystis* sp. PCC 6803 had reduced levels of PS I (27) and fewer thylakoid membranes. However, the authors of that study concluded that the reduction in thylakoid membrane content affected the ratio of PS I and PS II. Other studies suggested that Vipp1 was essential because it played a major role in the biogenesis of thylakoid membranes (4, 24). However, based on the results obtained in this study, precisely the opposite is the case in *Synechococcus* sp. PCC 7002. Vipp1 is clearly required for the biogenesis of PS I, and the absence of PS I leads to a greatly decreased level of thylakoid membranes in *Synechococcus* sp. PCC 7002 cells.

In contrast to previous studies in *Synechocystis* sp. PCC 6803, a fully segregated null mutation was constructed in the *vipp1* gene of *Synechococcus* sp. PCC 7002 in this study, and we were able to complement this mutant heterologously in *trans* with the *Synechocystis* sp. PCC6803 *vipp1* gene to restore the wild-type phenotype. These experiments clearly demonstrate that the function of Vipp1 is highly conserved, but Vipp1 is clearly not required for viability in the cyanobacterium *Synechococcus* sp. PCC 7002. Since 1996, the genomes of many cyanobacteria have been sequenced (*e.g.* see Refs. 28 and 29). Comparative analysis of these genomic data shows that some cyanobacteria, such as certain *Prochlorococcus* species, lack the *vipp1* gene. However, these cyanobacteria clearly still assemble thylakoids and produce functional PS I complexes (30). Thus, Vipp1 is not required for the formation of thylakoid membranes in *Prochlorococcus* spp.; nor is it essential in all cases for PS I biogenesis. These observations suggest that cyanobacteria must have redundant mechanisms to assemble PS I, probably PS II, and thylakoids. This is almost certainly one of the reasons why it has been so difficult to establish mechanistic details for the biogenesis of PS I and PS II.

It has been reported that reduced expression of Vipp1 in *Synechocystis* sp. PCC 6803 resulted in a decreased PS I content and an altered PS I/PS II ratio, reduced thylakoid content, and a reduced percentage of trimeric *versus* monomeric PS I complexes (27). Mutants lacking phosphatidylglycerol (PG) synthase, encoded by the *pgsA* gene, in *Synechocystis* sp. PCC6803 are not viable unless PG is added to the growth medium (23). When cells of a *pgsA* mutant were deprived of PG over many days, a phenotype similar to that for Vipp1 depletion was noted with respect to PS I complexes. Depletion of PG not only led to decreased PS I activity but also caused a depletion of PS I trimers and an increase in PS I monomers. However, PsaL was still inserted into membranes and could reassemble trimeric complexes in the absence of protein synthesis when PG was added back to cells (23). The crystal structure of trimeric PS I complexes from *Thermosynechococcus elongatus* showed that three PG molecules are tightly associated with each monomeric PS I complex and thus may play a role in PS I biogenesis (2). An *A. thaliana* mutant strain unable to synthesize PG was no longer able to grow photoautotrophically and had a severe reduction in Chl and thylakoid membranes (31), which could potentially be due at least in part to a loss of functional PS I complexes. Collectively, these results suggest that a relationship exists among Vipp1, PG biosynthesis, and biogenesis of PS I complexes and that collectively Vipp1, PG synthesis, and PS I biogenesis strongly influence thylakoid membrane structure and biogenesis.

Analysis of the *trans*-complemented *vipp1* mutant and the *psaAB* deletion mutant strains further indicated that Vipp1 is

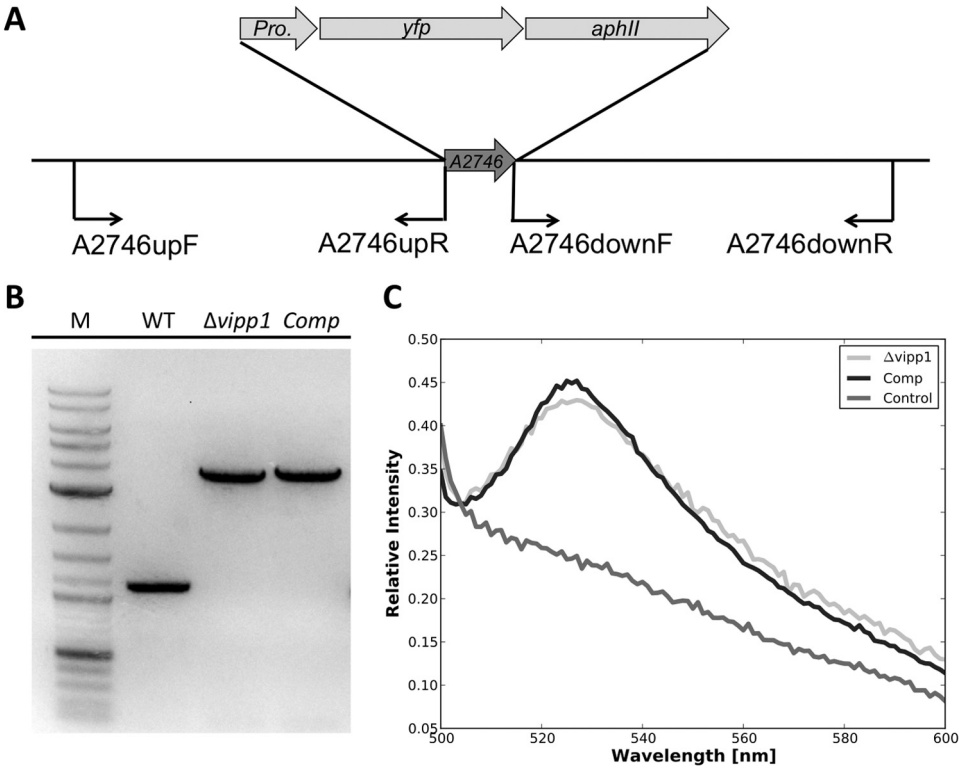

FIGURE 11. **Construction (*A*) and verification (*B*) of insertion of P_{psaAB} or P_{chlLN} promoters fused to *yfp* into a neutral site in the chromosome of *Synechococcus* sp. PCC 7002 strains and relative YFP fluorescence emission in the resulting strains (*C*).** *A*, scheme showing the replacement of open reading frame SynPCC7002_A2746 by *yfp* reporter gene fusions by homologous recombination and selection with kanamycin (*aphII* gene cassette confers resistance to kanamycin). Promoter regions (*Pro.*) for *psaAB* or *chlLN* were transcriptionally fused to the *yfp* gene, which was placed upstream from an *aphII* gene, which encodes aminoglycoside phosphotransferase II and confers resistance to kanamycin. *B*, agarose gel electrophoresis of PCR amplicons using primer set A2746upF and A2746downR to verify the introduction of the promoter-*yfp-aphII* constructions in the respective strains. Template DNAs came from the WT strain, the *vipp1* mutant, and the Comp. *C*, fluorescence emission spectra showing YFP emission in the constructed reporter strains. The control spectrum shows fluorescence emission from the *vipp1* strain, which does not contain the *yfp* gene. Very similar levels of YFP fluorescence emission were detected in the *vipp1* and Comp strains, which carried the *yfp* gene fused to the P_{psaAB} promoter. These data indicate that Vipp1 does not modify transcription from the P_{psaAB} promoter.

more likely involved primarily in the biogenesis of PS I complexes rather than thylakoid membrane biogenesis, because PS I complexes and activity are restored to wild-type levels in the *trans*-complemented *vipp1* mutant strain. In contrast, in the *vipp1* null mutant, no PS I complexes accumulated, and no PS I activity was detected. Previous studies also suggested that a critical Vipp1 concentration might be required for thylakoid membrane protein complex formation (27). Vipp1 has been reported to form rodlike structures *in vivo* (32), and it is possible that these Vipp1 structures could assist in the translation, transport, and/or insertion of membrane-associated subunits into thylakoids. Thus, the loss of Vipp1 might interfere with one or more of these processes and therefore interfere with the insertion of the PsaA and PsaB polypeptides into the thylakoid membranes. Although it seems clear that the loss of Vipp1 interferes with one or more of these processes, it certainly is not yet clear at which level Vipp1 acts to interfere with PS I biogenesis. However, the reduced level of *psaAB* transcripts in *vipp1* mutant cells suggests that Vipp1 probably acts directly or indirectly at the level of translation (see below).

In *Synechococcus* sp. PCC 7002, a *psaAB* deletion mutant lacking PS I had normal transcript levels for *vipp1* but greatly reduced thylakoid membrane content, and similarly, the *vipp1* mutant that lacks PS I had reduced thylakoid membrane content. Restoration of PS I levels in cells caused thylakoids to return to wild-type levels and overall thylakoid structure, which implies that PS I plays an important role in the formation of structurally normal and functional thylakoid membranes. These observations generally agree with observations that depletion of PS II had only minor effects on thylakoid membrane formation (33) and that intracytoplasmic membranes were much less abundant in a mutant depleted of both PS I and PS II (34). It has been suggested that PS I plays a role in the early steps to form thylakoid membranes and that PS II is then involved in forming highly ordered tubular structures of thylakoid membranes together with PS I (34). A direct interaction of Vipp1 with Albino3.2 protein in *Chlamydomonas reinhardtii* also implicated Vipp1 in the integration of thylakoid membrane proteins (35). The thylakoid-localized protein, Albino3.2, belongs to the conserved YidC/Oxa1p/Alb3 protein family, and it plays an essential role during the insertion of photosystem reaction center polypeptides (such as PsaA, PsbA (D1), and PsbD (D2)) into the thylakoid membranes (35, 36). Overproduction of Vipp1 occurred when Albino3.2 was depleted in *C. reinhardtii* (35). This observation suggests that Vipp1 is involved in stabilizing the membrane structure during the Albino3.2-mediated protein insertion into thylakoid membranes and that Vipp1 may deliver photosystem polypeptides to Albino3.2 for insertion into thylakoid membranes. If PsaA and PsaB were cotranslationally inserted into thylakoids during or

after Vipp1 action, this could explain why transcripts for *psaAB* were reduced in the *vipp1* mutant. Transcript levels for *yidC* were similar in *Synechococcus* sp. PCC 7002 cells in the presence or absence of Vipp1 (see supplemental Table S1). Furthermore, we observed no changes in transcript levels for genes encoding other PS I polypeptides, PS I-specific chaperones (*e.g. rubA*, *ycf3*, *ycf4*), or other general chaperones or proteases. Correspondingly, the transcript levels for all of these components were similar in the wild type, the *vipp1* mutant, the complemented *vipp1* mutant strain, and the *psaAB* deletion strain.

Abundant lipid bodies were detected in the *vipp1* mutant strain, which suggests that Vipp1 is not directly involved in lipid synthesis and accumulation. However, the abnormal localization of lipids in *vipp1* mutant indicates that Vipp1 directly or indirectly affects the insertion of lipids into thylakoid membranes. Previous studies suggested that Vipp1 played a stimulatory role in the cpTat transport system, potentially by enhancing protein binding interactions with lipid-rich regions of thylakoid membranes (37). Interestingly, it appears that lipid synthesis still occurs, but normal lipid insertion into membranes is apparently greatly reduced when PS I complexes are not inserted into the membranes in *Synechococcus* sp. PCC 7002. As observed previously in other organisms, it appears that there is a relationship between membrane biogenesis, lipid insertion, and PS I complex biogenesis and membrane insertion and that Vipp1 is necessary for all of these processes to proceed normally (24).

Nordhues *et al.* (38) recently studied the role of Vipp1 in *C. reinhardtii* by RNA interference. They found that core complexes for PS I and PS II as well as the cytochrome b_6f complex and ATP synthase were reduced 14–20% in Vipp1-depleted cells, but light-harvesting complex II levels increased by 30%. These authors proposed a highly speculative hypothesis that Vipp1 provides structural lipids for the biogenesis of some protein complexes of the thylakoid membrane. However, the obvious differences in their results in *C. reinhardtii* and those presented here for *Synechococcus* sp. PCC 7002 strongly suggest that Vipp1 may play different roles in prokaryotes and eukaryotes. Alternatively, redundant pathways may exist in some but not all organisms that could explain the substantial phenotypic differences observed in different organisms.

Transcription profiling suggested that Vipp1 might function as a potential transcriptional regulator of the *chlLN* and *psaAB* operons. The phage shock protein A (PspA), a key transcription regulatory protein in bacteria (39), has significant sequence similarity with Vipp1 and is thus is structurally related to Vipp1 as well. In other studies, the translocation of proteins by TAT complexes in *Escherichia coli* was blocked in a *pspA* mutant, but this phenotype could be relieved by expression of the *vipp1* gene from *Synechocystis* sp. PCC 6803 in the mutant (40). These findings indicate that cyanobacterial Vipp1 can functionally replace bacterial PspA. Subsequent studies suggested that the α-helical, PspA-like domain of Vipp1 plays a crucial role in forming high molecular mass complexes (41). Phylogenetic analyses of Vipp1 and PspA clearly suggest that these proteins form separate clades (24), and most cyanobacteria contain a protein from each family. A possible explanation for these results is that Vipp1 evolved from cyanobacterial PspA by gene duplication and may have gained a novel function in cyanobacteria in thylakoid biogenesis or perhaps stress responses. In *Synechocystis* sp. PCC 6803, *vipp1* expression increased under high salt conditions (42), and in *C. reinhardtii*, *vipp1* expression increased under high irradiance (43). These findings suggest that Vipp1 may play a role in responses to these stress conditions, although its expression might also increase if PS I levels increase under these same conditions. However, our results with reporter strains clearly showed that Vipp1 is not directly involved in the transcriptional regulation of either the *psaAB* or *chlLN* genes of *Synechococcus* sp. PCC 7002. We propose that *psaAB* transcripts decreased because of an effect of Vipp1 on translation or co-translational insertion of these PS I polypeptides into the thylakoid membrane. The decreased transcript levels for *chlLN* are probably due to the reduced demand for Chl *a* in the Vipp1 mutant, which does not accumulate PS I (Table 1). It is also interesting that the marine symbiont strain UCYN-A, which lacks PS II and cannot fix CO_2 but has retained PS I complexes (44), has also retained two copies of the *vipp1* gene like *Trichodesmium erythraeum*, another nitrogen-fixing marine cyanobacterium.

In conclusion, our results show that Vipp1 is not essential for viability of the cyanobacterium *Synechococcus* sp. PCC 7002 and that it is most likely involved in the biogenesis of PS I, possibly by participating in the insertion of PS I polypeptides into thylakoid membranes. Our results further suggest that normal thylakoid membrane biogenesis is dependent upon assembly of PS I but is not directly dependent on Vipp1. Further studies to elucidate the precise role of Vipp1 in PS I and lipid insertion into membranes will enhance our knowledge of the underlying mechanisms of thylakoid membrane biogenesis and photosynthetic protein assembly.

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